A STUDY OF MICROBIOLOGICAL PROFILE IN OBSTRUCTIVE PULMONARY DISEASE IN A TERTIARY CARE HOSPITAL IN THANJAVUR

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M.D.(MICROBIOLOGY)

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY OF MICROBIOLOGICAL PROFILE IN OBSTRUCTIVE PULMONARY DISEASE IN A TERTIARY CARE HOSPITAL IN THANJAVUR"** is a bonafide record done by **DR. M. LIDWIN MARY**, during the period of her Post graduate study from April 2016 to September 2017 under the guidance and supervision in the Institute of Microbiology, Thanjavur Medical College Hospital, Thanjavur, in partial fulfillment of the requirement for **M. D. MICROBIOLOGY** Degree Examination of the Tamilnadu Dr. M. G. R. Medical University to be held in May 2018

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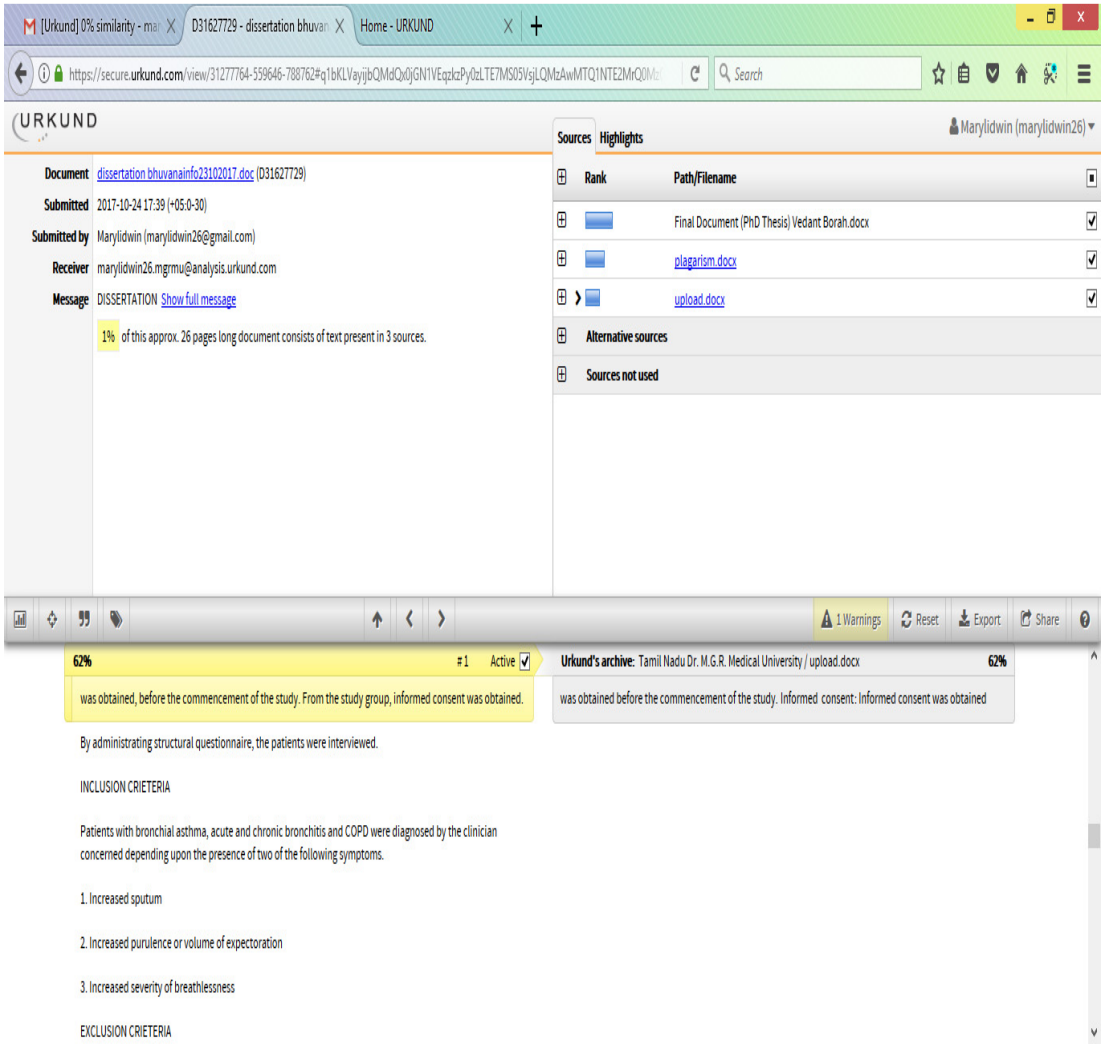

By administrating structural questionnaire, the patients were interviewed.

INCLUSION CRIETERIA

Patients with bronchial asthma, acute and chronic bronchitis and COPD were diagnosed by the clinician concerned depending upon the presence of two of the following symptoms.

1. Increased sputum

2. Increased purulence or volume of expectoration

3. Increased severity of breathlessness

EXCLUSION CRIETERIA

CERTIFICATE – II

This is to certify that this dissertation work titled **"A STUDY OF MICROBIOLOGICAL PROFILE IN OBSTRUCTIVE PULMONARY DISEASE IN A TERTIARY CARE HOSPITAL IN THANJAVUR"** of the candidate **DR. M. LIDWIN MARY** with registration Number **201514202** for the award of the degree of **M. D.** in the branch of **MICROBIOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from Introduction to conclusion pages and result shows **1** percentage of plagiarism in the dissertation.

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DECLARATION

I declare that the dissertation entitled **"A STUDY OF MICROBIOLOGICAL PROFILE IN OBSTRUCTIVE PULMONARY DISEASE IN A TERTIARY CARE HOSPITAL IN THANJAVUR"**submitted by me for the degree of M.D. is the record work carried out by me during the period of April 2016 to September 2017 under the guidance of **DR.EUNICE SWARNA JACOB**, Head of the Department of Microbiology, Thanjavur Medical College, Thanjavur. This dissertation is submitted to the TamilnaduDr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of the degree of M. D. Microbiology (Branch IV) Examination to be held in May 2018.

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TABLE OF CONTENTS

A STUDY OF MICROBIOLOGICAL PROFILE IN OBSTRUCTIVE PULMONARY DISEASE IN A TERTIARY CARE HOSPITAL THANJAVUR.

ABSTRACT

BACKGROUND AND OBJECTIVES

COPD is a chronic slowly progressive disorder, characterized by airways obstruction emphysema, and chronic bronchitis, often clinically grouped together and referred to as chronic obstructive pulmonary disease, damages both the acinar level (emphysema) and bronchial level (bronchitis). Because of increase in smoking, environmental pollutants and other noxious exposures, the incidence of COPD has increased leading to significant morbidity and mortality. Bacterial infections are the most commonobservable cause of acute exacerbation in COPD. Acute exacerbation is characterized by increase in cough, dyspnoea and sputum production. The aim of our study is to find out the prevalence of microbial pathogens in COPD patients, with special reference to antibiotic susceptibility and their resistance pattern from hospital data.

SETTINGS AND DESIGN

It was a prospective study carried out at the Institute of Microbiology, Thanjavur Medical College in association with the Department of Thoracic Medicine at Thanjavur Medical College Hospital, Thanjavur from April 2016 to September 2017.

MATERIALS AND METHODS

The study population consists of 100 patients admitted in Thoracic Medicine Unit presenting with signs and symptoms of COPD. All the respiratory samples were subjected to direct gram staining, culture, biochemical reaction and the isolates were identified according to standard techniques. Antibiotic sensitivity was done by Kirby-Bauer method according to CLSI standard**.**

RESULTS

COPD was common in the age-group of 40-60. Tobacco smoking was strongly associated with the study group. Corpulmonale 46% was the most common complication. Positive bacteriological culture was obtained in 43% of cases. Mixed infection among COPD patients was found in 10% of culture positive cases. Sputum purulence was significantly correlated with culture positivity.

The commonest organism in the respiratory samples in COPD patients were gram negative bacteria 86% as compared to gram positive bacteria 14%. Among gram negative organisms *Klebsiella pneumoniae* 43% was the most commonly and significantly isolated organism followed by *Pseudomonas aeruginosa*. Prevalence of extended spectrum β lactmase 43%.Multi drug resistance in *Pseudomonas aeruginosa* 19%. Presence of MRSA 50%. Among the fungal isolates *were Candida albicans* 41%, *Aspergillus* niger 23%, Aspergillus flavus 18% and *Aspergillus fumigates* 18%. *Haemophilus influenza* and *Mycoplasma* were not isolated.

CONCLUSION

To conclude,gram negative bacteria were more frequently isolated in our patients. Early antimicrobial treatment depending on the antimicrobial sensitivity leads to reduction of increasing burden of antibiotic resistance.

INTRODUCTION

COPD is defined as a disease state characterized by airflow obstruction that is not fully reversible. It is usually progressive in nature associated with an abnormal inflammatory response of the lungs to chronic inhalational exposure from smokes, dusts and other air pollution. The physiological changes in the airways are due to loss of elasticity and recoil due to cigarette smoke.[2-3]

COPD is a leading cause of morbidity and mortality world-wide. The W. H. O. estimated that COPD is currently the sixth leading cause of death and disability world-wide. It is estimated that COPD will become the third leading cause of death world-wide by 2020 and fifth leading cause of disability[4-5].

Microbial infections in respiratory tract are most frequent than any other organ and account for the largest number of work-days lost in the general population; account for 20-40% of out –patient; and 12-35% of in-patient attendance in a general hospital[5-7], and COPD is a leading cause of morbidity&mortality.

Microbial infections can result whenever the defence mechanism of normal lungs are impaired. That is, alterations produced in the bronchial epithelium by the damaging action of smoking favour bacterial adhesion, and

colonization leads to chronic infections that contribute to progressive pulmonary damage (or) whenever the resistance of the host in general is lower, which includes chronic diseases, immunological deficiencies, treatment with immunosuppressive agents, leukopenia and usually virulent infection[2].

The natural history of COPD is characterized by frequent exacerbations with an increase of cough, purulent sputum production and breathlessness. Majority of exacerbations are infectious in etiology. Childhood infections are probably a risk factor for the development of COPD. Chronic infections may lead to recurrent chronic inflammation for chronic bacterial colonization of the airways. It can potentially predispose to loss of lung function as well as increase the risk of exacerbation[8,9].

Three classes of pathogens responsible for acute exacerbations of COPD by infecting lower respiratory tract are respiratory viruses, aerobic gram-positive and gram-negative bacteria and atypical bacteria[4].

Patients with frequent exacerbations appear to lose lung function at an accelerated rate and exacerbations of COPD increase the rates of hospitalization and mortality and decrease the quality of life and increase economic burden[8].

This condition is highly serious in our country. Air pollution and smoking are the highest risk factors in India which are the main causes of COPD that lead to increase in microbial infection[12].

Empirical treatment was given for more than 80% of patients without proper investigation. So the effectiveness of treatment is decreased due to emerging new resistant strains leading to recurrent microbial infections[4].

In our hospital we also noticed an increasing incidence of microbial infections in COPD patients. This led to conducting a prospective study in order to record clinical and microbiological observations and find out the bacterial and fungal etiology and anti-microbial sensitivity pattern of microbial infections in COPD patients so that anti-microbial treatment could be started early depending on the anti-microbial sensitivity results, that is important for better prognosis of disease and improve the quality of life of the patient.

AIMS AND OBJECTIVES

1. To detect pathogenic organism, by microscopic examination of sputum samples.
2. To isolate and identify aerobic bacteria and fungal agent causing microbial infections in COPD patients.
3. To study the incidence of gram negative bacteria among the bacterial isolates
4. To study the incidence of mycoplasma among the bacterial isolates
5. To study the incidence of fungal agent causing COPD
6. To determine antimicrobial susceptibility pattern of the various bacterial isolates in COPD patients.
7. To determine the molecular characterization of the commonest isolated organism by polymerase chain reaction.

REVIEW OF LITERATURE

Pulmonary disease is the first clinical presentation and most frequent complication of COPD. And the most common manifestation of pulmonary disease is lower respiratory tract infection, particularly COPD of bacterial etiology.

Incidence of microbial infections are increased, whenever the defence mechanism of normal lung is impaired and with a degree of immunosuppression.

Smoking, ambient air-pollution and occupational exposure lead to increased risk of COPD.

The lung defence mechanisms

Can be interfered with many factors , such as the following:-

1. Loss (or) suppression of cough reflex
2. Injury to mucocilliary apparatus
3. Interference with the phagocytic (or) bactericidal activity of alveolar macrophages
4. Pulmonary congestion and edema
5. Accumulation of secretion leading to bronchial obstruction.

EARLY HISTORICAL LANDMARKS OF COPD

The foundation of modern knowledge of the pathologic anatomy of pulmonary emphysema was laid by J. Gough in 1952[12]

The appearance of enlarged respiratory air spaces on the surface of human lungs was first illustrated by Ruysch in 1961[5]

In 1967, the Swiss physician Bonnet referred COPD as "Voluminous lung" and in 1769, the Italian anatomist *Giovanni Morgagni* reported lungs were "turgid" particularly from air[3].

Followed by Mathew Baille in 1807 not only provided an earliest illustration and brief description of emphysema, but also pointed out its essentially destructive character.

In 1814, the British physician Charles Badham became the first to use the term bronchitis[14]

In 1821, *Rine Laennac* known as father of chest medicine had described the relationship between emphysema and chronic bronchitis[13]

The spirometer which is key to the diagnosis and management of COPD was invented by John Hutchinson in 1846.

In 1965 it was thought that Dr.William Brisco was the first person to use the term COPD.

HISTORICAL BACKGROUND OF BACTERIAL INFECTIONS

In 1885 the German – Austrian paediatrician *Theodor Escherich* discovered *Escherichia coli* [17]

Klebsiella pneumonia also called *Friedlander's bacillus* was described in 1882 by a German microbiologist and pathologist Carl *Friedlander. K.pneumoniae* is best known as a pathogen of the human respiratory system[20]

The genus is named by the German physician and bacteriologist Edwin Klebs. In 1882 *Carle Gessard* a chemist and bacteriologist from Paris, France discovered *pseudomonas aeruginosa.*

Sir Alexander Ogston, a British surgeon discovered *staphylococcus*[16]

MYCOPLASMA PNEUMONIAE

Mycoplasma pneumoniae was first discovered by Pasteur, who called it a pleuropneumonia - like organism[22]

Later in 1898 Nocard and Roux isolated a micoplasma species in culture from bovine[24].

Borrel in 1910 named these organisms as Asterococcus Mycoides[21]

Later in 1929 Nowak placed them under the term mycoplasma. It was first isolated in tissue culture from the sputum of a patient with primary atypical pneumonia by Eaton *et al* in 1944, and thereafter it became known as the Eaton agent[25]

In 1961 Marmionanf Goodbum postulated that the Eaton agent was a PPLO and not a virus[27]

MYCOLOGY

In 1839 Langenbeck identified an illness (typhoid fever) associated with the presence of a fungus[23]

Berg in 1841 evidenced that thrush is caused by a fungus[23]

Audry in 1887 evidenced that the organism isolated from cases of thrush was a single kind of fungus, *candida albicans*, the form it takes depending on its growth medium.

At the third international congress of microbiology in 1939,Martin adopted Christine Bearhout's use of the genus *Candida* for nine species.

Martin's reclassification was done in 1940.

Aspergillus was named by Pier Antonio *Micheli*, an Italian priest and biologist in1729[28]

In 1863, the species *fumigatus* was first described by a physician George W.Frensenius.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

The patient with diffuse pulmonary disease can be put in one of two categories[2]

- ⇨ Obstructive Disease (airway disease)
- ⇨ Restrictive Disease

Obstructive Disease –

Characterized by an increase in resistance to airflow, owing to partial or complete obstruction at any level from the trachea and large bronchi to terminal and respiratory bronchioles.

Restrictive Disease –

Characterized by reduced expansion of lung parenchyma, with decreased total lung capacity.

The major diffuse obstructive disorders are:-

Emphysema, chronic bronchitis and asthma.

- ⇨ Expiratory airflow obstruction may result either from anatomic airway narrowing, classically observed in asthma or from less of elastic recoil of the lung, which characteristically occurs in emphysema.

DEFINITION

COPD is defined as a disease state characterized by airflow obstruction due to chronic bronchitis or emphysema. The airflow obstruction which is generally progressive may be accompanied by airway reactivity and may be partially reversible[2-3]

Although asthma (reversible airway hyperactivity) is a distinct disorder, it may be a component of COPD in some patients.

COPD is associated with abnormal inflammatory response of the lungs to chronic inhalations exposure from smokes, dusts and other air pollutants.

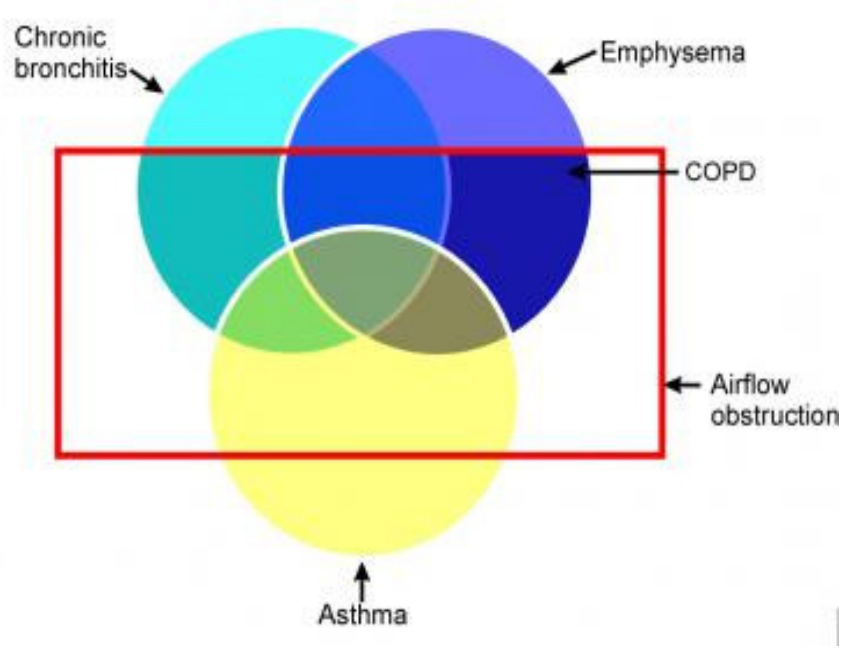
Chronic Bronchitis[2]

Is defined as the presence of a chronic productive cough on most days for 3 months, in each of two consecutive years, in a patient, in whom other causes of chronic cough have been excluded.

Emphysema –[2]

It is defined as abnormal, permanent enlargement of the distal airspaces distal to the terminal bronchioles accompanied by destruction of their walls and without obvious fibrosis.

Snider has popularized the use of a **Venn Diagram** that shows the relationships between chronic bronchitis, emphysema and asthma .



RISK FACTORS[8,12]

1. Tobacco smoking – accounts for 80 – 90% of the risk of developing COPD
2. Childhood lung infection – less than 14 years of age (bronchitis, pneumonia or whooping cough)
3. Passive smoking – environmental tobacco smoking.
4. Genetic consideration – Severe α_1 Antitrypsin deficiency with a proven genetic risk factor for COPD. PiZ allele increase is the most common form of severe Alpha – AT deficiency

5. Air pollution

- working in high foggy areas
- coal mining, steel mill
- industrial pollutions

6. Indoor air pollution – rural indian women exposed to domestic fuels using bio-massfuel

7.Occupation – several occupational exposure including coal mining, gold mining and cotton textile dust have been suggested as risk factors for chronic air-flow obstruction.

8. Men affected more than women (increased smoking in men)

9. Increased age

10. Low socio-economic status

11. Chronic broncho-pulmonary infection – which causes airway damage and progressive airway obstruction

12. Growth and nutrition – impaired growth in utero may be a risk factor for the development of chronic respiratory disease

13. Atopy and airway hyperresponsiveness(AHR)_is associated with an asthmatic predisposition with associated allergic pneumonia in which AHR may be important in the pathogenesis of persistent airway obstruction.

CAUSES OF MICROBIAL INFECTIONS IN COPD

a) INFECTIOUS AGENTS

1) Respiratory viruses

a) Rhino virus

b) Corona virus

c) Influenza virus

d) Para influenza virus

e) Adeno virus and respiratory syncytial virus.

2) Aerobic gram positive and gram negative bacteria

3) Atypical bacteria

b) ENVIRONMENTAL FACTORS

1. Includes change in temperature
2. Humidity
3. Air pollution
4. Occupational exposure
5. Smoke exposure
6. Noxious gases and
7. Chemical irritants

c) HOST FACTORS

1. Patients with low socio economic status
2. Nutritional and immune deficiencies
3. Poor personal hygiene
4. Lack of exercise and
5. Life style modification

MICROBIAL ETIOLOGY OF COPD

INFECTION	COMMON	LESS COMMON
BACTERIA	Streptococcus pneumoniae Hemophilus influenzae Staphylococcus aureus Pseudomonas aeuroginosa	Moraxella Legionella Nocardia
MYCOPLASMA	Mycoplasma pneumoniae	Mycoplasma Pneumonia M.fermentants M. pirum M. penetrans M. genitallium
FUNGI	Pneumocystis carnii Histoplasma capsulatum Cryptococcus neoformans Aspergillus species	Candida albicans Pencillium mareneffei Blastomyces dermatitidis

BACTERIAL INFECTIONS IN COPD

Bacteria has been isolated from the sputum in approximately 60% of exacerbations of COPD. The most common organisms isolated include *H influenza ,Haemophilus parainfluenzae, S.pneumoniae and M.catarrhalis*

Most virulent organisms in the airways of severe chronic bronchitis patients with acute exacerbations include, *staphylococcus aureus, pseudomonas species* and members of *enterobactericiae family*[4]

Atypical pathogens such as *mycoplasma* and *Chlamydia species* seem to increase the risk of exacerbations

Last atypical organisms and viruses can cause primary infections that can lead to severe airway inflammation enabling a secondary increase in bacterial proliferation that can lead to exacerbations [33]

RESPONSIBLE BACTERIA IN ACUTE EXACERBATIONS OF CHORNIC BRONCHITIS[37]

a) COMMON BACTERIAL PATHOGENS (30 – 50%)

- Hemophilus influenza
- Hemophilus parainfluenza
- Streptococcus pneumoniae
- Moraxella catarrhalis

b) LESS COMMON BACTERIAL PATHOGENS (10-15%)

- Pseudomonas aeruginosa
- Enterobactericiae other gram negative bacilli
- Staph aureus and other gram positive cocci

C) ATYPICAL PATHOGENS (5-15%)

- Chlamydia pneumoniae
- Mycoplasma pneumoniae

Among the gram negative bacilli mucoid forming isolate klebsiella is a predominant one and pseudomonas is also becoming dominant

INCIDENCE OF BACTERIAL INFECTIONS IN COPD

Bacterium is a common cause of infection in COPD patients in a study by Timothy F murphy. Majority of them were gram negative organism. 65% showed positive culture for bacteria in a study by Bari mar *et al.*

The organisms that commonly play pathogenic role in acute exacerbation of COPD are *pseudomonas and klebsiella, Acinetobacter*, *Moraxella Catarrhalis and Enterobactericiae also* contributed in exacerbation of COPD.

The prevalence of P.*aeruginosa* infection in AECOPD is estimated to the 4%. But increase to as much as in 13% patients, with advanced airway obstruction in a study by Lawra Martinez *et al*.

P.*aeruginosa is* isolated from sputum samples from 4-15% adults with COPD.

INCIDENCE OF MYCOPLASMA IN COPD

80% of the cases of acute exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) have a infective etiology, atypical bacteria including mycoplasma pneumonia accounting for 5-10% of these[61]

There are two main subtypes of *M.pnuemoniae* frequently isolated from clinical specimens (*su et al* 1990)

Atypical pathogens reference Laboratory Database attributes 12% range (11-15%) of global CAP incidence to *M.pnueumoniae* .(Arnold *et al* 2007)

Mycoplasma infection in India has been reported for 35% patients with community acquired pneumonia in both children as well as in adults[48]

INCIDENCE OF FUNGAL INFECTIONS IN COPD

Fungal infections have emerged as a world–wide health care problem in recent years, owing to extensive use of broad-spectrum antibiotics, long term use of immunosuppressive agents, increasing use of hyperalimentation and indwelling devices and the increasing population of terminally ill, debilitated and immunocompromised patients.

In recent years, Chronic Obstrutive Pulmonary Diseases (COPD) have been recognized as a risk factor for invasive aspergillosis which occur as a result of smoking tobacco or long term exposure to pollution caused by burning of wood and other biomass fuels[49]

Airway colonization by *Aspergillus* species is a common feature of Chronic pulmonary disease.

Increased prevalence of *Aspergillus* species colonization was observed in COPD patients,although other fungal infections such as colonization of *Pnemocystis jiroveci* are also possible.

C.albicans(60.5%) was the most frequently recoverd species by culture, followed by *C.trophicalis(16%), C.krusei(15.5%)* and *C.globrata(8%)*.

1.63% of COPD patients had *Aspergillus* species in their lower respiratory tract samples and 22.1% of these patients had probable IPA by Guiner *et al*.

The most common species of filamentous fungi was *Aspergillus* species. *A.fumigatus(43.7%) A.flavus(31.2%), A.niger(6.2%) and, A.terreus (6.2%)*

Huerta et al reported that the isolation rate of *Aspergillus* species from sputum species was 16.6%. In addition, Kurhade *et al* and Shahid *et al* isolated *Aspergillus* species from sputum samples of 16.3% and 14.&% of the cases with chronic respiratory disease.

Candida *albicans* was the most frequent isolate being recovered from 42.9% of patients, followed by *Aspergillus flavus(21.4%),Aspergillus* fumigates (14%) and *Aspergillus niger (10.7%).*[60]

MORTALITY AND MORBIDITY OF COPD

Nowadays, incidence of COPD has increased, so that in India, the occurrence of this disease has increased by 4.1%

It was found that COPD was the most common underlying disease (22%) in critically ill patients by Khasawnath *et al*.

Chronic Obstructive Pulmonary Disease is a major cause of mortality and morbidity across the globe.

According to World Health Organization estimates, 65 million people have moderate to severe COPD. More than 3 million people died of COPD in 2005 Corresponding to 5% deaths globally. So it is estimated to be the third leading cause of death by 2030.[62]

There are 30 million COPD patients in India.

India contributes a significant and growing percentage of COPD mortality estimated to be amongst the highest in the world i.e., more than 64.7 estimated age standardized death rate per 100,000 amongst both sexes[62]

The reported prevalence estimates have ranged from 2 - 22% in men and from 1.2 to 19% in women.[63]

The national burden was estimated to be 14.84 millon[6]

PATHOLOGY (PATHOLOGICAL CHANGES IN COPD)[1]

Cigarette smoke exposure may affect the large airways (2 mm diameter) and alveolar space changes in large airways cause cough and sputum, while changes in small airways and alveoli are responsible for physiological alterations.

LARGE AIRWAY

- Cigarette smoking often results in mucous gland enlargement and goblet cell hyperplasia
- These changes are proportional to cough and mucus production that define chronic bronchitis
- Goblet cells, not only increase in number but in extent throughout the bronchial tree
- Patients may have smooth muscle hypertrophy and bronchial hyper reactivity leading to airflow limitation
- Neutrophil influx has been associated with purulent sputum of upper respiratory tract infections that hamper patient with COPD

SMALL AIRWAYS

- The major site of increased resistance in most individuals with COPD is always 2 mm diameter.

- Characteristic cellular changes include goblet cell metaplasia and replacement of surface secreting clara cells , with mucus-secreting and infiltrating mononuclear inflammatory cells
- Reduced surfactant may increase surface tension at the air- tissue interface, predisposing to airway narrowing (or) collapse
- Loss of bronchiolar attachments, as a result of extra cellular matrix destruction, may cause airway distortion and narrowing in COPD

LUNG PARENCHYMA

- Emphysema is characterized by destruction of gas-exchanging airspaces (or) the respiratory bronchioles, alveolar ducts and alveoli.
- These walls become perforated and latter obliterated with coalasence of small distant airspaces intora abnormal and much larger airspaces.
- Emphysema is classified into distinct pathological types, the most important being centriacinar and pan acinar.

(A) CENTRIACINAR EMPHYSEMA (CENTRI LOBULAR)

Is more common than the panacinar type, constituting more than 95% cases. This type most frequently associated with cigarette smoking often in association with chronic bronchitis is characterized by enlarged airspaces found in associated respiratory bronchioles (central (or) proximal part of the acini).

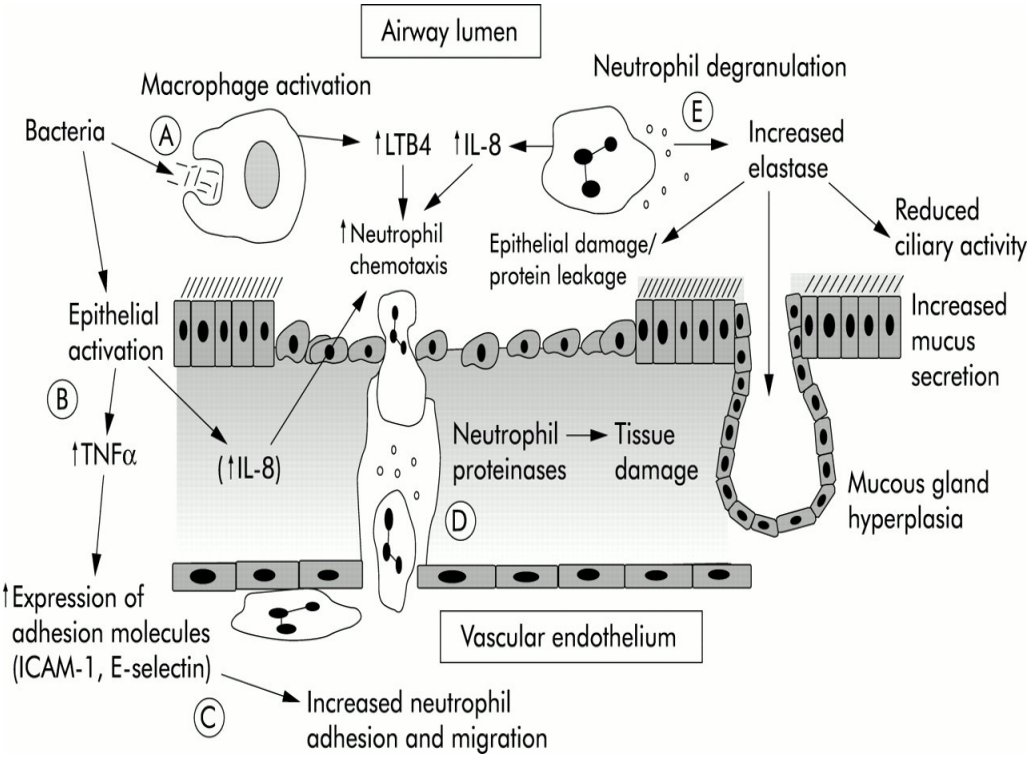
B. PANACINER EMPHYSEMA (PAN LOBULAR)

In this type, the acini are uniformly enlarged from the level of the respiratory bronchiole to the terminal blind alveoli .“ pan’’ refers to the entire acinus, tends to occur more commonly in the lower zones usually more severe at the bases.

This type of emphysema is associated with (α-AT) deficiency .

PATHOGENESIS[1]

The Elastase-Anti elastase hypothesis–Elastin, the principal component of elastic fibres, highly stable component of the extracellular matrix, that is critical to the integrity of both the small airways and the lung parenchyma.



Patients with genetic deficiency in α AT, the inhibitor of the serine protease neutrophil elastase were at increased risk of emphysema.

Inflammation and extracellular matrix proteolysis, upon exposure to oxidants from cigarette smoke, resulting in transcription of matrix metaloproteinase-9, proinflammator cytokines, interleukin-8 and tumour necrosis factor α leads to neutrophil recruitment Matrix metalloproteinases and serine proteinases most notably neutrophil elastase, work together by degrading the inhibitor of the other, to lung destruction.

These three collagenases (MMP-1,MMP-8 and MMP-13) that initiate cleavage of interstitial collagens are also induced in both inflammatory cells and structural cells in COPD.

Ineffective repair of elastin and perhaps other extracellular matrix components result in airspace enlargement that defines pulmonary emphysema.

PATHOPHYSIOLOGY[1]

Persistent reduction in forced expiratory flow rates is the most typical finding in COPD.

Increase in the residual volume and the residual volume/total lung capacity ratio, non uniform distribution of ventilation and ventilation-perfusion mismatching also occur.

Airflow obstruction

Patient with airflow obstruction, related to COPD have a chronically reduced ratio of FEV1/FVC.

Hyperinflation

In COPD, there is often "air trapping", increased residual volume and increased ratio of residual volume to total lung capacity and progressive hyperinflation. Hyperinflation helps to compensate for airflow obstruction.

Gas exchange

Pulmonary hypertension cause corpulmonale and right ventricular failure due to COPD occurs, in those individuals who have marked decrease in FEV_1 (<25% of predicted) together with chronic hypoxia (PaO_2<55mmHg)

Non uniform ventilation and ventilation perfusion mismatching are characteristic of COPD-accounts for essentially all of the reduction in PaO_2 that occurs in COPD

MICROBIAL INFECTIONS IN COPD[53]

Potential pathways by which microbes could contribute to the course and pathogenesis of COPD identified.

1. Childhood lower respiratory tract infection impair lung growth, and is reflected in smaller lung volumes in adulthood.
2. Microbes cause a substantial proportion of acute exacerbations of chronic bronchitis which cause considerable morbidity and mortality.
3. Chronic colonization of the lower respiratory tract by microbial pathogens, amplifies the chronic inflammatory response present in COPD and leads to progressive airway obstruction (vicious circle hypothesis)
4. Microbial pathogens invade and persist in respiratory tissues, alter the host response to cigarette smoke (or) induce a chronic smoke imflammatory response and thus contribute to the pathogenesis of COPD.
5. Microbial antigens in the lower airway induce hypersenstivity and enhances airway hyperreactivity and induce eosinophilic inflammation.

FREQUENT EXACERBATIONS HAVE

1. Impaired health status
2. Reduced physical activity levels
3. Increased lower airway bacterial colonization
4. Accelerated lung function decline

STAGING OF COPD(3)

Depends on factors such as

- ⇨ Arterial blood gas levels
- ⇨ Time and distance walked
- ⇨ Sensation of dysponea and
- ⇨ Body mass index into the following

a) ATS criteria

Stage 1 – $FEV_1 \geq$ 50 percent of predicted

Stage 2 - $FEV_1$35 to 49 percent of predicted

Stage 3 - $FEV_1<$ 35 percent of predicted

b) ERS criteria

Mild – $FEV_1 \geq$ 70 percent of predicted

Moderate - FEV_1 50 to < 80 percent of predicted

Severe - FEV_1 < 50 percent of predicted

c) GOLD criteria (See later for clinical severity)

Stage 1 $FEV_1 \geq$ 80 percent of predicted

Stage 2 FEV_1 30 to < 80 percent of predicted

Stage 3 FEV_1 < 30 percent of predicted

SPIROMETRIC CLASSIFICATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Stage I : mild	$FEV_1/FVC < 0.70$
	$FEV_1 \geq 80$ percent predicted
Stage II : moderate	$FEV_1/FVC < 0.70$
	$50\% \leq FEV_1 < 80\%$ predicted
Stage III : severe	$FEV_1/FVC < 0.70$
	$30\% \leq FEV_1 < 50\%$ predicted
Stage IV : very severe	$FEV_1/FVC < 0.70$
	$FEV_1 < 30\%$ predicted or FEV_1 < 50% predicted plus chronic respiratory failure.

Patients with stage 1 COPD usually do not have severe hypoxemia, and arterial blood gas analysis is not required. For stage 2 and 3 arterial blood gas measurements should be done.

Most patients of COPD will be of stage 1 type. Patients of this stage with complaint of dyspnoee, should be investigated further. Stage 2 patients are only a minority. Stage 3 patients also include a minority of COPD cases and need specialized care.

RESPIRATORY FAILURE:

Arterial partial pressure of oxygen (Pa_{02}) < 8.0 Pa (60mm Hg) with or without arterial partial pressure of CO_2 (Pa_{CO2}) > 6.7 kPa (50 mm Hg) while breathing air at sea level. The characteristic symptoms of COPD are chronic and progressive dyspnoea, cough, and sputum production. Chronic cough and sputum may precede the development of airflow limitation by many years. This pattern offers a unique opportunity to identify smokers and others at risk for COPD, and to intervene when the disease is not yet a major health problem. Conversely, significant airflow limitation may develop without chronic cough and sputum production.

Stage I: Mild COPD: Characterized by mild airflow limitation ($FEV_1/FVC < 0.70$, $FEV_1 \geq 80$ predicted). Symptoms of chronic cough and sputum production may be present, but not always. At this stage, the individual is usually unaware that his or her lung function is abnormal.

Stage II: Moderate COPD: Characterized by worsening airflow limitation ($FEV_1/FVC < 0.70$, $50\% \leq FEV_1 < 80\%$ predicted), with shortness of breath typically developing on exertion and cough and sputum production sometimes also present. This is the stage at which patients typically seek medical attention because of chronic respiratory symptoms or an exacerbation of their disease.

Stage III: Severe COPD: Characterized by further worsening of airflow ($FEV_1/FVC < 0.70$, $30\% \leq FEV1 < 50\%$ predicted)

CLINICAL PRESENTATION OF COPD PATIENTS

SYPMTOMS

1. Cough with expectoration
2. Sputum is usually mucoid purulent (or) mucopurulent depending upon the superimposed infection.
3. Increase in breathlessness
4. Chest pain
5. Fever
6. Weight loss
7. Increased fatigue

PHYSICAL SIGNS

1. Dyspnoea
2. Tachypnoea
3. Tacycardia
4. Expiratory wheeze
5. Fine inspiratory crepitation

Signs of hyperinflation

1. Barrel shaped chest with kyphosis
2. Increase dantero posterior diameter and prominent sternal angle
3. Flat diaphragm and reduced chest wall movement

In later stages

1. Emaciation
2. Cyanosis-visible in lips and nailbeds
3. Reduced jugular venous pressure
4. Polycythemia
5. Edema

COMPLICATIONS

1. Pneumonia
2. Corpulmonale
3. Type1 respiratory failure
4. Type2 respiratory failure
5. Right ventricular failure
6. Secondary polycythemia
7. Cardiacfailiure
8. Respiatory acidosis and coma
9. Pneumothorax
10. Sleep abnormalities
11. High incidence of depression
12. Comorbidities –coronary artery disease,deep venous thrombosis and pulmonary embolism

DIAGNOSIS OF COPD

Include history, physical findings and laboratory findings associated with microbiological investigations.

Laboratory findings include:

1. Haematological tests-
    - Hb estimation,
    - total leukocyte count,
    - differential count,
    - ESR,
    - blood glucose level,
    - blood urea ,
    - Serum creatinine
2. Pulmonary function tests
3. Radiological studies
4. Altered blood gas analysis
5. Serology-typically performed by isoelecric focusing of serum,which reflects the genotype at the p1 locus for the common alleles
6. PCR-molecular genotyping of DNA can be performed for the common PI alleles(M,S and Z)

MICROBIOLOGICAL INVESTIGATIONS

Are used to isolate and identify the organisms and to determine antimicrobial susceptibility pattern of the bacterial isolates.

Different types of samples are collected from lower respiratory tract to perform microbial investigations.

The most common samples are:

A. NON INVASIVE

1. Expectorated sputum

The most common sample and the least invasive, collected under direct supervision was sent to the laboratory for culture in lower respiratory tract infections

2. Induced sputum

Induction of sputum was done using a nebulizer with 3% hypertonic saline for 15minutes and sputum was collected in sterile wide mouth containers.

3. Gastric lavage

- in uncooperative patients

- in other disorders like neurological disorders and coma

B. MINIMALLY INVASIVE

Endotracheal aspirate

C. INVASIVE

1. Bronchial washings

2. Bronchoalveolarlavage (direct aspiration of secretion, through a bronchoscope)

3. Lung tissue

1.MICROSCOPY

Direct microscopy examination

Helps in determining the quality of specimen and in predicting the likely pathogens by characteristic appearance

2. STAINING METHODS [54,55,56]

a. Gram stain

b. Acid fast stain

3. ANTIGEN DETECITION

For detection of atypical pathogens;

a. DIF

b. Capture ELISA

a. Direct immunofluorescence test;

- detects the mycoplasma antigen directly in the clinical specimens.

b.Capture ELISA Assay

- is available using monoclonal antibodies against *P1adhesion* Antigen,

4. SEROLOGY

For detection of atypical pathogens

Specific antibody detection test

1. Immunofluoresence assays
2. Latex agglutination assays
3. ELISA using protein 𝒫1 antigen

Non specific antibody detection tests;

1. Cold agglutination test : it uses human O blood group RBC(I antigen) and test is carried out at 4°C

5. POLYMERASE CHAIN REACTIONS

a) Multiplex PCR

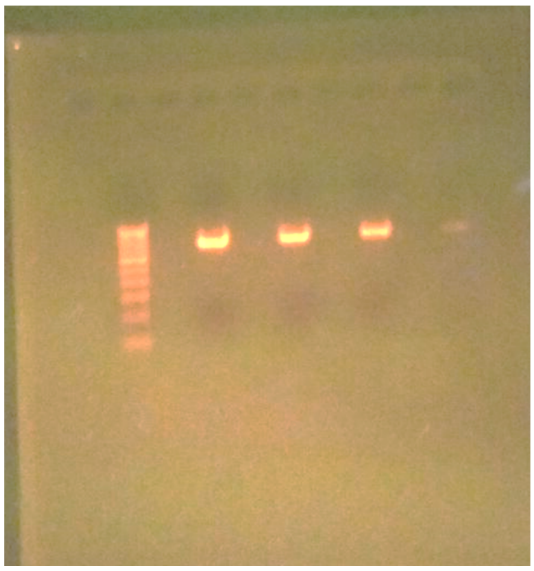
b) Real time PCR

PCR is an important technique , which gives confirmatory results and higher diagnostic yield along with conventional diagnostic methods.

Identification of the Isolates

Identification of the isolated strains was based on colony morphology, microscopic observation. The samples were sub cultured in LB broth and centrifuged to obtain cell pellet. To identify the isolated microorganism 16S rRNA gene was amplified with universal primers as a forward primer AGA GTT TGA TCC TGG CTC AG and as a reverse ACG GCT ACC TTG TTA CGA CTT. Sequencing analysis was carried out using AB Life sciences 3500 genetic analyser. The sequence data was further processed using online tool ncbi BLAST. https://www.ncbi.nlm.nih.gov/.

Bacterial DNA after Amplification



Sample 1: *Klebsiella pneumoniae* strain Amm2 16S ribosomal RNA gene, partial sequence accession no: KJ950284.1

Sample 2: *Escherichia coli* strain ETEC 16S ribosomal RNA gene, partial sequence accession no: MF919609.1

Sample 3: *Staphylococcus aureus* strain HZW450 chromosome, complete genome accession no: CP020741.1

Sample 4: *Pseudomonas aeruginosa* strain ET05 16S ribosomal RNA gene, partial sequence accession no: MF398395.1

BLAST ® » blastn suite » RID-YGXZS5C2014

BLAST Results

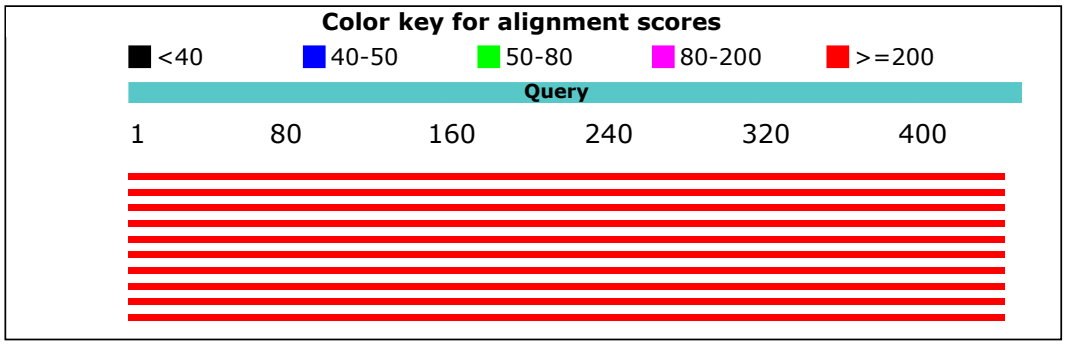
Job title: Nucleotide Sequence (440 letters)

RID YGXZS5C2014 (Expires on 10-20 18:01 pm)

Query ID	lcl|Query_16051	**Database Name**	nr
Description	None	**Description**	Nucleotide collection (nt)
Molecule type	nucleic acid	**Program**	BLASTN 2.7.0+
Query Length	440		

Graphic Summary

Distribution of the top 10 Blast Hits on 10 subject sequences



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Klebsiella pneumoniae strain Amm2 16S ribosomal RNA gene, partial sequence	813	813	100%	0.0	100%	KJ950284.1
Klebsiella pneumoniae strain FD28-2 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	MF767580.1
Klebsiella pneumoniae strain lsy1 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	KR269873.1
Klebsiella pneumoniae strain Amm1 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	KJ950283.1
Klebsiella variicola strain R25 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	KM019906.1
Klebsiella sp. 2359 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	JX174236.1
Klebsiella pneumoniae strain LSRC119 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	JF772079.1
Klebsiella sp. YSI6A 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	JQ624587.1
Klebsiella pneumoniae strain sctccT53 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	HQ622344.1
Klebsiella pneumoniae strain sctcc295 16S ribosomal RNA gene, partial sequence	808	808	100%	0.0	99%	HQ622341.1

Alignments

Klebsiella pneumoniae strain Amm2 16S ribosomal RNA gene, partial sequence
Sequence ID: **KJ950284.1** Length: 1503 Number of Matches: 1
Range 1: 1 to 440

Score	Expect	Identities	Gaps	Strand	Frame
813 bits(440)	0.0()	440/440(100%)	0/440(0%)	Plus/Plus	

Features:

```
Query  1    AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGC  60

Query  61   GGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGA  120

Query  121  AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCA  180

Query  181  AGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGGTTAGCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGGTTAGCT  240

Query  241  GGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCA  300

Query  301  GCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG  360

Query  361  CACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGT  420
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Sbjct  361  CACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGT  420

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            ||||||||||||||||||||
Sbjct  421  AAAGCACTTTCAGCGGGGAG  440
```
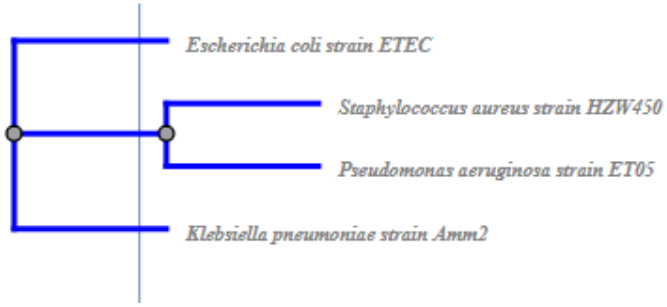



PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (1). *Taq* DNA Polymerase is an enzyme widely used in PCR (2). The following guidelines are provided to ensure successful PCR using NEB's *Taq* DNA Polymerase. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb may require further optimization.

Protocol

Reaction setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C).

Component	25 µl reaction	50 µl reaction	Final Concentration
10X Standard *Taq* Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng

Taq DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR
Nuclease-free water	to 25 µl	to 50 µl	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes from ice to a PCR machine with the block preheated to 95°C and begin thermocycling.

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

General Guidelines:

1. Template:

   Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	Amount

genomic	1 ng–1 µg
plasmid or viral	1 pg–1 ng

2. Primers:

   Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg^{++} and additives:

   Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with *Taq* DNA Polymerase. The final Mg^{++}concentration in 1X Standard *Taq* Reaction Buffer is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$.

   Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (3) or formamide (4).

4. Deoxynucleotides:

   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. *Taq* DNA Polymerase Concentration:

   We generally recommend using *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

6. Denaturation:

   An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer initial denaturation of 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 95°C is recommended.

   During thermocycling a 15–30 second denaturation at 95°C is recommended.

7. Annealing:

   The annealing step is typically 15–60 seconds. Annealing temperature is based on the T_m of the primer pair and is typically 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m. The NEB Tm Calculator is recommended to calculate an appropriate annealing temperature.

   When primers with annealing temperatures above 65°C are used, a 2-step PCR protocol is

possible (see #10).

8. Extension:

   The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C is recommended.

9. Cycle number:

   Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

10. 2-step PCR:

    When primers with annealing temperatures above 65°C are used, a 2-step thermocycling protocol is possible.

    Thermocycling conditions for a routine 2-step PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 65-68°C	15-30 seconds 1 minute/kb
Final Extension	65-68°C	5 minutes
Hold	4-10°C	

11. PCR product:

    The PCR products generated using *Taq* DNA Polymerase contain dA overhangs at the 3´–end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

References:
1. Saiki R.K. et al. (1985). *Science*. 230, 1350-1354.
2. Powell, L.M. et al. (1987). *Cell*. 50, 831-840.
3. Sun, Y., Hegamyer, G. and Colburn, N. (1993). *Biotechniques*. 15, 372-374.
4. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990). *Nucleic Acids Res.*. 18, 7465.

TREATMENT[56]

Important goals particularly against bacterial infections are

1. Improve pulmonary function
2. Relief of symptoms
3. To reassess the cause of disease to reduce the risk of further exacerbation.

Antibiotic treatment should always aim on

Decreasing bacterial load,

- To decrease airway inflammation
- To decrease work of breathing and
- To select appropriate antibiotic therapy

is essential to control microbial infections.

Development of antimicrobial resistance is by far the most important one. New emerging strains develop resistance pattern to old classes of antibiotics-lead to determine sensitivity pattern.

The antibiotics should be chosen as per patients' affordability, severity of exacerbation, bacterial spectrum and have the knowledge of local bacteriological profile. Among them, the prevalence of MRSA, ESBL, Amp C and MBL producers should be important.

Multidrug resistant organisms are resistant to three (or) more group of antibiotics with different mechanisms of action.

Inadequate empiric therapy lead to emergence of multidrug bacteria (MDR).Although *pseudomonas aeuroginosa and methicillin resistant staphylococcus aureus* (MRSA) are the two common organisms, other MDR bacteria includes extended – spectrum beta lactamases (ESBL) containing gram negative bacilli—ESBL, AmpC, MBL and penicillin resistant streptococcus pneumonia.

1. BETA LACTAMASES IN GRAM NEGATIVE BACILLI:[64]

a) EXTENDED SPECTRUM BETA LACTAMASES (ESBL)

ESBL's are included in Bush class A β lactamases capable of hydrolyzing Pencillins, Oxyimnocephalosporins and Monobactams (Aztreonam) and inhibited by β lactamase inhibitors (Clavulanic acid, Sulbactam, Tazobactam) but have no detectable activity against Cephamycins or Carbapenems (Imipenem, Meropenem)

Produced mainly by members of family *enterobacteriaceae* (*Klebsiella and E.coli,*) and some nonfermentors.

These enzymes are carried on plasmid and may spread rapidly from strain to strain, can be transferred between gram negative rods, even of other species.

Often mediate resistant to other antimicrobial agents, such as aminoglycosides, tetracycline and sulfonamides.

Recent surveys suggest that 50-70% 0f exacerbations are due to respiratory infections; 10% due to environmental pollution and upto30% are of unknown etiology.

Study for monitoring antimicrobial resistance program 2007 stated 42.2% were ESBL positive.

Ecoli isolates were 42.2% positive and also 35.8% of *k.pneumonia* isolates.

METHODS FOR DETECTION OF EXTENDED SPECTRUM BETALACTAMASES

1. Screening methods: using cefotaxime / ceftriaxone cefpodoxime/ ceftazidime / cefepime 30microgram discs by disc diffusion method should be followed by confirmatory methods.
2. CLSI phenotypic confirmatory methods by both broth microdilution method and disc diffusion method.
3. Double disc testing(DDT)
4. ESBL E test.
5. Automated methods.(MAST DISC TEST, Vitet card)
6. Molecular detection methods.

2.AmpC BETA LACTAMASES[65]

Amp C βlactamases are Bush class C β lactamases (chromosomal or plasmid mediated) that degrades most cephalosporins and are resistant to all beta lactamases (including Cephamycins) and are not blocked by beta lactamase inhibitor (Clavulanate, sulbactam or tazobactam) combinations except Carbapenems.

They can be differentiated from ESBL by their ability to hydrolyze cephamycins.

Multidrug resistant plasmid mediated Amp Cenzymes,seen in *Klebsiella spp , E.coli , salmonella* and *p.mirabilis*.[65]

Studies from various parts of India have reported the prevalence of Amp C in clinical isolates of enterobacteriace as varying from 2.2% to 20.7% [65]

DIAGNOSTIC TESTS FOR AmpC DETECTION:

1. Screening methods using cefoxitin disc by disc diffusion method,

   Organisms showing resistance to cefoxitin (zone size<18mm)should be considered as probable Amp C producer, and should be followed by confirmatory tests.
2. Cefotetan DDT
3. E test AmpC Strips contain cefatetan on one end and cefotetan-cloxacillin on the other end.
4. Molecular methods

   Multiplex PCR to detect plasmid mediated resistance using six primers (or) Amp C gene promoter sequencing to detect chromosomal promoter mutations.

3. METALLO BETA LACTAMASES IN GRAM NEGATIVE BACILLI: [66]

MBL are Bush class B β lactamases capable of hydrolyzing carbapenems by production of metallo beta lactamases. Carbapenem are the most potent agent for the treatment of multidrug resistant gram negative bacterial infections.

In recent years, there has been an increase in the prevalence of carbapenem resistance which is acquired metalo β lactamases(MBL) and found mainly in pseudomonas aeurginoa spp and Enterobactericaeae. Resistance to carbapenems may be due to impermeability through cell wall due to loss of omrDporin, up regulation of an active efflux system, carbapenems production, or production of MBLs.

The prevalence of imipenem resistance (carbapenems production) was observed in 40.3% isolates of *acinetobacter spp* and in 40-54 % of *pseudomonas* [66]

DETECTION OF MBL

1. Screening methods: using carbapenem disc (Doripenem, Ertapenem, Imipenem and meropenem), followed by
2. Confirmatory methods: Imipenem – EDTA DDT
3. Modified Hodge test (MHT).
4. MBL E test-imipenem on one end of the strip, imipenem+EDTA on the other end
5. Molecular methods-PCR techniques using multiplex PCR detection for carbapenemes and sequencing of carbapenamase genes.

4. METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

MRSA is a major pathogen responsible for both hospital and community acquired infection. These are β lactamases, which are clinically resistant to all available β lactamases including penicillin, cephalosporins, β lactam/β lactamase inhibitor combinations, monobactam and carbapenems.

The incidence of MRSA varies from 25% in western part of India to 50% in South India. Sangeetha *et al* 2010

β lactamases produced resistance by

1. High level of β lactamases production
2. Modification of normal penicillin biniding proteins (PBPs) and
3. Presence of acquired penicillin binding protein (PBP_2)

Resistant mechanisms in MRSA are by high level of β lactamase production (BORSA) or production of modified PBP_2 (MOD-PBP) with decreased drug affinity

The vast majority of clinical strains that exhibit this type of resistance have acquired the mecA gene that encodes PBP2a which has decreased affinity for binding the compound.

DETECTION METHODS FOR MRSA

1. Screening method-Routine disc diffusion procedure with cefoxitin disc (30µg)/oxacillin,followed by
2. Confirmatory method-broth microdilution screen usuing the CLSI breakpoint for S.aureus
3. E-test
4. Molecular detection of mecA gene using either a commercial latex agglutination test (or)

   Molecular detection methods detected PBP_2.

Other PCR MRSA detection assays also used other staphylococcal gene markers –femA, femB, Sa442 and orfX.

MATERIALS AND METHODS

This prospective study was conducted at the Institute of Microbiology, Thanjavur Medical College in association with Thoracic Medicine Department.

STUDY PERIOD

One year from April 2016 to September 2017

STYDY POPULATION

The study population consisted of 100 patients, older than 18 years, admitted in Thoracic Medicine unit presenting with signs and symptoms of COPD.

ETHICAL CLEARANCE

Institutional Ethical Committee approval was obtained, before the commencement of the study. From the study group, informed consent was obtained. By administrating structural questionnaire, the patients were interviewed.

INCLUSION CRIETERIA

Patients with bronchial asthma, acute and chronic bronchitis and COPD were diagnosed by the clinician concerned depending upon the presence of two of the following symptoms.

1. Increased sputum

2. Increased purulence or volume of expectoration

3. Increased severity of breathlessness

EXCLUSION CRIETERIA

1. Outpatient status

2. Evidence of bronchiectasis (or) pnemoniae, malignancy(or)severe immunosuppression and need for mechanical ventilation

3. Absence of an adequate sputum specimen(inappropriate sputum)

4. Treatment with any antibiotic within 24 hours before admission

DATA COLLECTION

Complete data about the patient's name ,age ,sex, hospital number, date of collection of sputum, history of present illness and symptoms were collected from the patient.

Past H/O smoking , occupational history was also collected.

Any history of exposure to indoor air pollution was collected from the female patients.

SAMPLE COLLECTION, TRANSPORT AND PROCESSING

Patients were educated about the difference between sputum and oral secretion. Early morning samples were obtained from cases that were clinically diagnosed as COPD. Patients were instructed to collect coughed sputum into a sterile wide mouth container with a screw cap after rinsing the mouth twice with plain water.

Sample containers were labeled after the collection and were transported to the laboratory immediately and processed within 30 minutes of collection.

SAMPLE COLLECTION

1. Expectorated sputum
2. Induced sputum (using a nebulizer with 3% hypertonic saline for 15minutes)

PROCESSING OF SPUTUM SAMPLES

Expectorated samples were used for isolation of bacterial and fungal pathogens.

SAFETY PRECAUTIONS

1. All sputum samples were potentially infectious and leak proof containers were used for collection and transportation of samples

2. Biological Safety Cabinet level 111 was used for carrying out all procedures involving sputum and protective wears like mask, gloves etc. were used.

3. Disinfection of the sputum containers by treating with freshly prepared 1-2% sodium hypochlorite solution and autoclaving was following.

The quality of expectorated sputum was assessed by macroscopic examination and microscopic examination.

Any sample that was thin, watery and with no purulent matter was considered unsuitable for further processing.

A. DIRECT MICROSCOPY

All the respiratory samples i.e., sputum were subjected to the following microscopic examination.

1. Direct gram stain

To detect the presence of bacterial cells, their gram reaction, morphology, arrangement and also to detect yeast cells.

a.Direct Gram Smear:-

The report had to include the following information.

- Whether many, moderate, few (or) scanty
- Gram reaction of the bacteria, whether gram positive (or) gram negative
- Morphology of the bacteria, whether cocci, Diplococci, streptococci, rods (or) coccobacilli.
- Also whether the organisms are intracellular
- Presence and number of pus cells.
- Presence of yeast cells and epithelial cells.

2. **Acid fast stain**

To detect the presence of mycobacterium tuberculosis bacilli in the sample.

3. **10% Potassium hydroxide mount**

To detect the presence of fungal elements.

B) HANGING DROP PREPARATION

Hanging drop preparation is one of the easiest methods to observe motility in a clinical microbiological laboratory.

Advantage of this method is that by this method, live bacteria can be observed. Examination of living organisms is useful to observe cell activities , viz.,

- Motility
- Binary fission
- Natural sizesand shapes of the cells.

Motility of Bactria

Can also be demonstrated by

1. Craigie's tube method
2. Swarming of the bacteria on a non-inhibitory medium (or) blood agar
3. Dark ground microscopy

Procedure:-

1. Take a clean grease free cavity slide.
2. Take a clean cover slip; apply paraffin to the four corners of cover slip.
3. Place a drop of broth culture on the cover slip, with the help of inoculating loop.
4. Place the cavity slide (cavity down) over the cover slip, so that the drop is placed in the center.
5. Invert the slide and observe under microscope.
6. First observe under low power (10X)

   Locate the edge of the drop, shift the focus to high power (40X) and observe.
7. Record the observation.

Sputum samples were mechanically homogenized with sterile glass beads using votex machine. All the sputum samples were prescreened with Gram stain, using Bartlett scoring system. Only those samples which met the acceptance criteria (a final score of > 0) were further processed for culture. Rest of the samples were discarded and repeat samples were obtainned in all possible cases.

BARTLETT SCORING SYSTEM ; BARTLETT'S GRADING SYSTEM FOR ASSESSING THE QUALITY OF SPUTUM SAMPLE: -

No of Neutrophils per 10X low – power field:-

	(Low – Power) Grade
< 10	0
10 – 25	+1
>25	+2
Presence of mucus	+1

No of epithelial cells per 10 X low power field

10 – 25	- 1
> 25	- 2

A final score of 0 or less indicates lack of active inflammation (or) contamination with saliva.

PREPARATON AND PROCEDURE FOR STAINING

1. **Gram's stain procedure**
   - Appropriate smear was made on a clean glass slide. The smear was fixed by passing the slide over flame 2-3times quickly.
   - The slide was covered with crystal violet solution and allowed it to act for 1 minute. Then the slide was rinsed with water.

- Holding the slide at an angle downwards pour on the Gram's iodine solution on the slide for one minute. Then rinse the slide with water.
- Pour a few drops of decolorizer to the smear (100% acetone) for 2to 3 seconds. Rense the slide immediately with water.
- Apply the counterstain for 1 minute, rinse with water , blot dry and then examine the smear under oil immersion microscopy.

2. Ziehl Neelson Staining Procedure;

Preparation of smear

- Select a new, unscratched slide and lablle with a laboratory serial number
- Make a smear, from yellow purulent portion of the sputum. Spread a good smear evenly, 2cmsx3cms in size and is neither too thin. The optimum thickness of the smear can be assessed by placing the smear on a printed matter, the print should be just readable through the smear.
- Let the smear air dry for 15-30 minutes.
- Fix the smear by passing the slide over the flame 3-5 times ,for 3-4 seconds each time.
- Place the fixed slide, on the staining rack with the smeared slide facing upwards.

Staining;

- Pour the smear with strong carbol fuchsin for 5 minutes with intermittent heating by flaming the underneath of the slide until the fumes appear.
- Allow the slide to cool for 5-7minutes.
- Gently rinse the slide with water to remove the excess carbolfuchsin stain.
- Decolourise the smear by adding 25% sulphuric acid for 3 minutes
- Counterstain the slide by pouring 0.1% methylene blue solution for 1 minute.
- Gently rinse the slide with water and tip the slide to drain off the water
- Place the slide in the slide tray and allow it to dry.
- Then examine it under oil immersion objective.

4. CULTURE:

The specimens were then plated into the following agar media.

Mac Conkey agar,5% Sheep bld agar, PPLO agar and Sabourauds dextrose agar.

All cultures were incubated at 37^{o}c under aerobic condition and in a carbon dioxide enriched atmosphere.

Plates were evaluated for growth at 24 and 48 hours and discarded after 5 days except for Sabouraud's dextrose agar that was kept up to 4 weeks.

- **MacConkey Agar**

1. Incubation

   Time – Upto 48 Hrs

   Temperature – $37^{o}C$

   Atmosphere – Ambient Air

2. Culture Reading – Daily

3. Pathogens That Grow – Enterobacteriaceae Non Fermenters

- **Blood Agar**

1. Incubation

   Time – Upto 48 Hrs

   Temperature – $37^{o}C$

   Atmosphere – 5-10% Co_2

2. Culture Reading – Daily

3. Pathogens That Grow – Streptococcus Species, Staph.Aureus, Moraxella Catarrhalis

- **Sabouraud Dextrose Agar**

1. Incubation

   Time – Upto 4weeks

   Temperature – $25^{o}C$ and $37^{0}C$

   Atmosphere – Ambient Air

2. Culture Reading – Daily For 1 week, twice weekly for next 3weeks

3. Pathogens that grow – Fungi

- **PPLO agar**

1. Incubation

   Time – Upto 4weeks

   Temperature – 25^{o}C and 37^{0}C

   Atmosphere – Ambient Air

2.Culture Reading – 2-3 days interval for 1 week, and weekly twice for the next 3weeks

3.Pathogens that Grow – Mycoplasma

Report General Growth character as under:-

(Depending on the medium used)

> General growth > colony characteristics > Surface characteristics

> Margin / Edge of colonies > optical characters

II. General growth

On blood agar – presence or absence of haemolysis and swarming

On Mac Conkey Agar – Presence or absence of lactose fermenting colonies

Colony Characteristics : < Size and shape

1. Size

   - the diameter in millimeters, after a specific duration of incubation.

2. Shape :

- punctiform, Rocend / Circular, Elliptical, Irregular, Fusiform

III. Surface characteristics

Of colonies can be described as

>smooth, > Contoured, >Radiate, > Concentric, > wrinkled

IV. Elevation Characteristics:-

> Swarming,>Flat, > Raised, >Convex, >Pulvinte, >Umblicate,

> Conical, >Umbonate

V. Margins / edges of colonies:-

Entire, Undulate, Lobate, Lacerate, Fimbriate, Cilate.

VI. Optical characters

1. Pigment Production (Describe)
   - Colour of the pigment
   - Whether pigment restricted to the colonies (insoluble pigment)
   - Whether pigment is dispersed in the medium surrounding colonies (soluble pigment)
2. Opacity
   - Transparent, Opalcent, Opaque, Dull / Matt, Glistening / Fluorescent / Mucoid

INTERPRETATION OF BACTERIAL CULTURES

The isolated colonies were identified by means of Garam's stain motility, catalase, oxidase test and coagulase test and by various biochemical reactions like Indole test, Metyhl red test, Voges Proskauertest Citrate utilization test Ureasetest Triple Sugar iron agar, Hugh Leifson's Oxidation fermentation test were performed .Sugar fermentation tests with sugars viz Glucose, Lactose, Sucrose, Maltose, Manose. Xylose, Arabinose and Dulcitol etc were done to identify the isolate according to Standard laboratory procedures.

IDENTIFICATION OF MYCOPLASMAS:-

1. DIENE'S STAIN PROCEDURE FOR

Mycoplasma colonies are easily identified by observing typical colonies. On agar medium visualization of colony morphology is facilitated by application of Diene's stain directly to the agar foreface.

Diene's stain: -

Is a non specific stain, that imparts a contrasting appearance of *mycoplasma* colonies. on agar, allowing easier visualization of colony morphology and characteristics.

Reagent: - Diene's Stain: -

Methylene Blue 2.5gms

Azure blue 1.2 gms

Maltose 10.0gms

$Na_2 Co_3$ 0.25 gm

Distilled water 100.0 ml

Procedure :-

1. Flood an agar plate containing *mycoplasma* growth with 1 ml of Diene's stain working solution.
2. Immediately rinse the agar surface with distilled water to remove the stain.
3. Decolorize the medium by adding 1 ml of 95% ethanol. Leave in contact with the agar for1 minute, then remove. Repeat the wash step a second time.
4. Rinse with distilled water and allow it to dry.
5. Observe the colonies under the low power of a microscope

2. RESULTS:-

INTERPRETATION

Mycoplasmas with the "Fried – egg" colony morphology will stain with a dark blue centre and light centre periphery and will appear highly granular. The agar background will be clear (or) slightly violet.

Mycoplasmas other then M *pneumoniae* will remain stained.

M *pneumoniae* reduces the methylene blue after a time and will become colorless

II. HEMADSORPTION TEST:

Among the respiratory *Mycoplama* , M *pneumoniae* is the only species, that will specifically absorb red blood cells.

This property, therefore, provides a method for the presumptive identification of M *pneumonia*.

When colony growth is noted on *mycoplasma* isolation media inoculated with respiratory tract specimens, a suspension of guinea pig erythrocytes is placed on the agar surface for a given time thenwashed off*M pneumoniae* colonies will adsorb some of the red cells to the colony surface.

Reagents:-

1. *Mycoplasma* glucose agar with suspicious coloumn present.
2. Washing guinea pig erythrocytes (0.2 – 0.4%) suspended in mycoplasma broth medium

III. Procedure:-

1. Flood the surface of agar with 2 ml of red cell suspension.
2. Incubate the plate at 35^0Cfor 30 minutes and rotate the plate occasionally to prevent the red cells from setting out.

3. Wash the surface of the plate three time with 3 ml of Mycoplasma broth by gently rotating the plate. Remove wash fluid by aspiration with a pipette.
4. Examine the colonies at 50 to 100 X magnifications under a dissecting microscope.

Positive test :- colonies with red cells adsorbed onto the surface – M. *pneumonia*

Negative test : - colonies with no red cells adsorbed – *Mycoplasma* species, not M. *pneumonia*.

III. GLUCOSE FERMENTATION

Sterile PPLO broth with 0-5% glucose with PH – 7.8 prepared as described above and about 2 ml of medium was dispensed in 13X 100 mm sterile screw capped tubes 0.2 ml of positive broth culture was inoculated and incubated aerobically at 37^{o} C for 10 days. Fermentation status was checked daily. A change in color from purple to yellow was noticed

IV. ARGININE HYDROLYSIS

Sterile screw capped tubes containing 2 ml of Arginine broth with 0.25% arginine at PH 7.0 was inaculated with 0.25 ml of broth culture of the isolates and inculcated aerobically at 37^{o}C for 10 days.

The result of hydrolysis was checked daily by observing a color change from purple to red.

V. UREA HYDROLYSIS:-

Ten percent of urea was incorporated into PPLO broth media at a final Concentration of 1 % in the media and PH of the medium was adjusted to 6.5 Screw capped tubes with sterile medium were inoculated 0.25ml of broth culture of the isolate and incubated aerobically at 35^{o}C for 10 days.

Urea breakdown was checked based on observing colour change from purple to red.

VI. SEROLOGICAL TESTS:-

About 2 ml of blood was centrifuged and serum was separated and commercially available *mycoplasma pneumoniae* IgM TMB ELISA (Bio Rad Laboratories) was done according to manufacturer's instructions applied in the kit.

IDENTIFICATION OF FUNGUS

9. Potassium Hydroxide Wet Mount Preparation

Procedure: -

1. Emulsify the specimen in a drop of 10 % KOH on a microscope slide with the help of a loop.
2. Apply gentle heat by passing the slide over a Bunsen flame for 3 – 4 times.
3. Cover the smear with the cover slip.
4. Leave it for 5 – 10 minutes.
5. Examine the slide under low(10X) and high power (40X) magnifications.
6. Examine the slide for 15 – 20 minutes for demonstration of shining fungal elements.

Results And Interpretations:-

Different fungi will have different morphological forms (yeasts, cells with pseudo hyphae, budding, septate, and aseptate, hyphae, granulesetc) which can be clearly seen in a KOH wet mount.

Interpretation of results should be done by critical analysis of the type, size and color of fungal elements which will be different for different fungi.

LACTOPHENOL COTTON BLUE (LPCB)

WET MOUNT PREPARATION:-

Tease mount preparation: -

Procedure: -

1. Place a drop of LPCB on a clean glass slide.
2. Remove a small portion of the colony and the supporting agar at a point between the centre and periphery and place it in the drop of LPCB.
3. With a needle, tease the fungal culture first and spread it in the LPCB.
4. Examine microscopically after giving sufficient time for the structures to take up the stain, usually 30 minutes.

Results And Interpretations:-

The fungal elements grown should be observed and results interpreted depending on the morphology of the hyphae and the spores.

Different fungi under LPCB wet mount will show different types of morphological structures including hyphae and the spores. This should be thoroughly differentiated and the fungus identified.

Fast growing fungi in case of slide culture preparation will give satisfactory results in 24 – 48 hours.

SLIDE CULTURE : -

Procedure:-

1. From the petri dish containing sabouraud's agar cut out one square cm block of agar for each slide culture to be inoculated.
2. With the flat side of a sterile bacteriological loop (or) with a sptula, place an agar block in the centre of the slide in the slide culture set up.
3. With a probe, inoculate around the periphery of the agar block, 3 – 4 fragments of the mould to be cultured.
4. With forceps, the tips of which have been flamed, place the cover slip on the agar block.
5. With a pipette, thoroughly moisten, but not to excess, the filter paper with sterile distilled water.
6. Incubate the slide culture at room temperature.
7. Remove the slide culture from the petri dish and dry the bottom of the slide with a tissue.
8. When growth appears beneath the cover slip, take a slide ,place a drop of LPCB on it, and place the cover slip removed from the block on the LPCB.
9. Place the slide on the microscope stage and examine.

The aerial hyphae including the conidiophores will be seen to grow along the undersurface of the cover slip.

Results And Interpretations:-

Small spore bearing fungi make beautiful permanent mounts, some large spore bearing organisms like Microsporumgypsium do not stain as well. With the type of hyphae arrangement of conidiophores, staining charactierticsetc, the final interpretation of the fungal type can be made.

GERM TUBE TEST

PROCEDURE

Take 0.5 ml of serum in a test tube and add half of a single colony to be tested by using a sterile loop, and mix with serum.

Incubate the tube at 37^{o} C for a maximum of 11/2 hrs.

Place one drop of suspension on to a slide and place cover slips over the drop.

Examine the slide under low (10X) and high power (40X) magnifications.

Observations:-

Under the microscope , the whole field under the cover slips is examined for any cell showing production of germ tube.

Germ tubes are seen as long tube like projections extending from yeast cells.

SABOURAUD'S AGAR MEDIUM FOR PRIMARY ISOLATION OF FUNGUS FROM CLINICAL MATERIALS:

PROCEDURE:

1. Weigh the ingredients
2. Dissolve the ingredients in water by gentle heating
3. Make up the volume of the medium to 1 litre.
4. Adjust the PH to 5.4
5. Add agar agar. Dissolve the agar by free steaming or by continuous stirring by placing the container in a water bath.
6. Filter through layers of gauze piece.
7. Distribute in bottles
8. Sterilize by autoclaving at 8 lbs pressure for 30 minutes.
9. Cool a part of sterile molten media at an approximate temperature of 52^{o}C keep remaining media in bottles
10. Distribute the part of cooled sterile medium in sterile petri dishes with sterile precautions'.
11. Store at 4^{o}C

SDA:

It is a selective medium for cultivation of fungi

- It contains glucose 40g
- Peptone 10g
- Agar 20 -30 g
- Distilled water – 100ml

It is pate, transparent slope dispensed in large text tubes with Ph 5.4

INTERPRETATION OF FUNGAL CULTURES

Inoculated SDA Slants were incubated at 25-30c for minimum of 4 weeks before discarding as negative. These Slants were inspected daily during the first week and twice weekly during the next three weeks for growth.

In case of yeasts, identification was done by Grams Stain morphology, germ tube test, morphology on cornmeal agar and biochemical tests by Standard microbiological techniques as recommended by CLSI.

Identification of filamentous fungi was done by preparing lactophenol cotton blue mount and studing the morphology of hyphae and conidial arrangement.

ANTIMICROBIAL TESTING;

Antimicrobial testing was done by disc diffusion method, using Kirby-bauer technique on Muller Hinton agar(High media),using appropriate antimicrobial drugs as CLSI guidelines.

Inoculated suspension, equivalent to a 0.5 Mac Farland Standard, incubated at 35+-2°C ambient for 16 to 18 hours.

Media and discs were tested for quality control using Standard ATCC control strains

The following standard strains were used:

1. Staphylococcucaureus-ATCC 25923
2. Eschericha coli-ATCC25922
3. Klebsiella pneumonia-(ESBL)-ATCC-700603
4. Pseudomonas aeuroginosa-ATCC 27853

The diameters of Zones of inhibition were interpreted according to CLSI Standards for each organism.

THE PANEL OF ANTIBIOTICS INCLUDED IN THE ANTI MICROBIAL SENSITIVITY TESTING FOR GRAM NEGATIVE BACILLI WERE;

Antibiotic	Disc content in µg	Diameter of Zone of Inhibition in mm Break Points		
		Sensitive	Intermediate	Resistant
Amikacin	30	≥ 17	15-16	≤ 14
Cefotaxime	30	>26	23-25	≤ 22
Ceftriaxone		≥ 23	20-22	≤ 19
Ceftazidime		≥ 21	18-20	≤ 17
Cortrimoxazole	1.25/23.75	≥ 16	11-15	≤ 10
Ciprofloxacin	5	≥ 21	16-20	≤ 15
Gentamicin	10	> 15	13-14	≤ 12
Imipenem	10	> 23	22-22	≤ 19
Piperacillin-Tazobactam	100/10	> 21	18-20	≤ 17

ANTIMICROBIAL SENSITIITY PATTERN OF GRAM POSITIVE AND GRAM NEGATIVE COCCI

Antibiotic	Disc content in µg	Diameter of Zone of Inhibition In mm Break Points		
		Sensitive	Intermediate	Resistant
Amikacin	30	≥ 17	15-16	≤ 14
Ampicillin	10	>17	-	≤ 16
Amoxy-cillinclavulanic acid	20/10	≥ 20	-	≤ 19
Cortrimoxazole	1.25/23.75	≥ 16	11-15	≤ 10
Ciprofloxacin	5	≥ 21	16-20	≤ 15
Cefoxitin	30	> 32	-	≤ 21
erythromycin	15	> 23	14-22	≤ 13
vancomycin	30	> 17	-	–

DETECTION OF β LACTAMASE ENZYMES PRODUCTION IN GRAM NEGATVE BACILLAI

A. EXTENDED SPECTRUM β LACTAMASES (ESBL) DETECTION METHODS

1. Screening method.

An inoculum of 0.5 McFarland Standard turbidity was prepared in a nutrient broth from isolated colonies taken from 18-24 hour agar plates. A sterile swab was dipped into the nutrient broth and inoculated into a dried and sterile Muller-Hinton Agar (MHA) plate. The antibiotics were applied to the surface of the plate after 3-5 minutes of inoculation. The discs were pressed firmly against the surface of the plate and distributed evenly so that the minimum distance was 24mm.

If any of the isolates had zone of inhibition for third generation cephalosporins - Ceftazidime 30µg≤22mm, Aztreonam 30µg ≤ 27mm, Cefotaxime 30µg ≤ 27mm and Cefriaxone 30 ≤ 25mm (ESBL breaking point) as well as strains which were resistant were taken as screen positive for ESBL.

All strains found to be ESBL Screen test positive were subject to further confirmation by CLSI.

2.PCDDT -Phenotypic Confirmatory Disc Diffusion Test

The Strains screened positive for ESBL production were tested by PCDDT for Confirmation, Discs of Ceftazidime (CAZ-30µg) and Ceftazidime with Clavulanic Acid (CAC-30/10µg) and Ceftaxime (CTX-30µg) and Cefotaxime-Clavulanic Acid (CTX/C-30/10µg) were dispersed at a minimum distance of 24mm on an MHA agar plate inoculated with the lawn culture of the isolate Screened positive for ESBL production and incubated aerobically at 37^0C overnight. If there was an increase in zone size by≥5mm with Ceftozidime/Clavulanic acid and Cefotaxime/Clavulanic acid in comparision to Ceftazidime or Cefotaxime alone, then the strain of *Enterobactericeae* was confirmed to be ESBL producer.

E .coli-ATCC 25922 and *KlebsiellaPneumoniae* ATCC-700603 were taken as controls for phenotypic confirmatory test for ESBL.

3. Double Disc Diffusion Synergy Test

In this test discs of third generation Cephalosporins and Augmentin (20µg/10µg) were kept 30mm apart from centre to centre on inoculated Muller-Hinton Agar (MHA).

A clear extension of the edge of the inhibition zone of Cephalosporin towards Augmentin disc was interpreted as positive for ESBL production.

B.Amp C –B-LACTAMASE DETECTIONS;

1. Screening Method

A 0.5 McFarland of the test isolate was swabed on MHA plate and disc of Cefotaxime (30µg), Ceftazidime(30µg) were placed adjacent to Cefoxitin (30µg) disc at a distance of 20mm from each other.

After incubation, isolates showing blunting of Ceftazidime or Cefotaxime zone of inhibition adjacent to cefoxitin disc or showing reduced susceptibility to either of the above drugs and cefoxitinwere considered as screen positive and selected for detection of AmpC β lactamases.

2.AmpC Disc Test;

A lawn culture of E.coli ATCC 25922 were prepared on MHA plate. Sterile disc (6mm) were moistened with sterile saline and inoculated with several colonies of test organism. The inoculated disc was then placed beside a Cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 35°C. A positive test appeared as a flattening or indentation of the of cefoxitin inhibition zone in the vicinity of the test disc. A negative test had an undistorted zone.

C.METALLO-β-LACTAMASES (MBL) DETECTION METHODS;

1.Imipenem-EDTA disc diffusion method;

A 10µg imipenem disc containing 750 mg of EDTA solution was used. The inhibition zone with imipenem –EDTA disc were ≤14 mm for the MBL-negative isolates and ≥17mm for MBL positive isolates.

Imipenem disc diffusion method was employed as a screening test to select suspected MBL strains showing resistance to imipenem which were further confirmed by imipenem –EDTA combined disc method and imipenem-EDTA double disc synergy test.

2. Imipenem-EDTA Combined disc Test

A lawn culure of test isolates was prepared. After allowing it to dry for five minutes, two imipenem dics, one with 0.5 EDTA and the other a plain imipenem disc, were placed on the surface of agar plates approximatetly 30mm apart.

The plates were incubated overnight at 37^{O}C. An increase in zone diameter of ≥7mm around imipenem+EDTA disc in comparision to imipenem disk alone indicated production of **MBL.**

DETECTION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS;

1.Disc diffusion method;

Colonies isolated from agar culture plates were suspended directly into broth to reach 0.5 McFarlands Standard.

A lawn culture of the staphylococcal isolates wsa made on the MHA plate and 30µg Cefoxitin discs was placed on the surface of lawn culture and incubated at 35c for 24 hours in ambient air.

According to CLSI criteria, isolates showing inhibition zone diameter ≥22mm were considered as methicillin sensitive strains and those that show inhibition zone diameter ≤21mm were considered as methicillin resistant zones.

Routine disc diffusion procedure with Cefoxitin disc (30µg) followed by confirmatory testing should be done using minimum inhibitory concentration(MIC) either broth microdilution

2. E-test.

Vancomycin E-test strips;

Have been used alone or in combination with a teicoplanin strip (Macro E test or MET)

RESULT

This study was conducted in Thoracic Medicine Ward of Thanjavur Medical College Hospital. The total number of patients admitted during the study period were 980 out of which 100 patients who fulfilled the study criteria were taken for this study.

TABLE 1

AGE AND SEX WISE DISTRIBUTION OF PATIENTS

Age	No of Patients	Percentage
<20	5	5
21-40 years	21	21
41-60years	47	47
>60 years	27	27

Chart

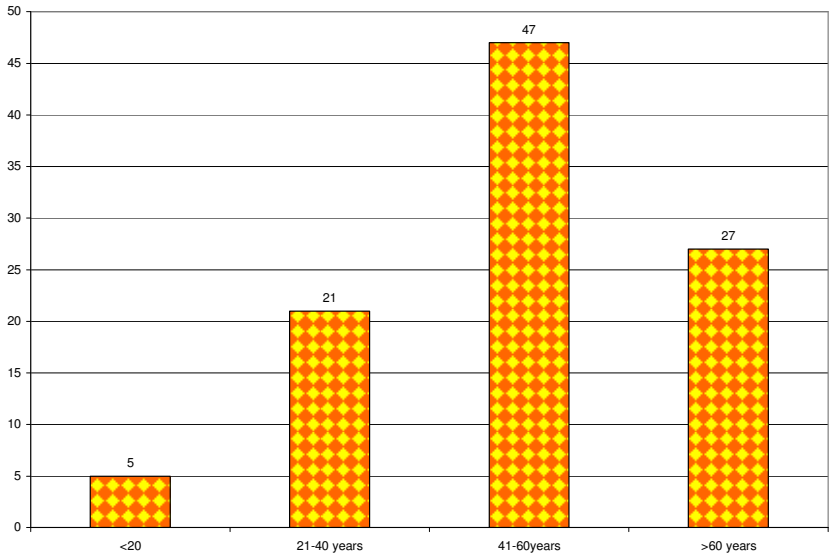


TABLE 2

SEX DISTRIBUTION OF PATIENTS

Sex	No of Patients	Percentage
Male	46	46
Female	54	54

Chart

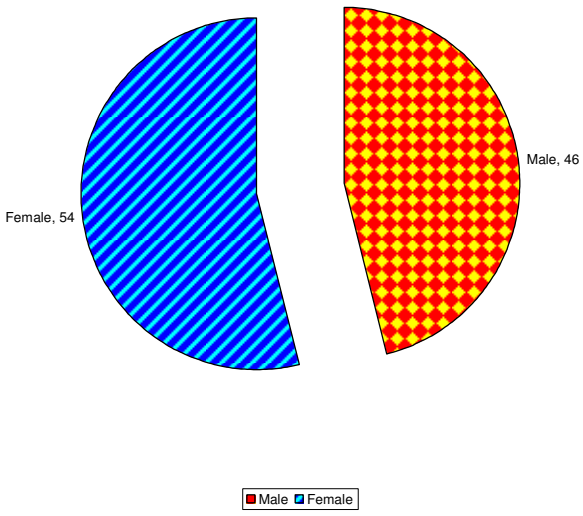


TABLE 3

AGE AND SEX GROUPS UNDER STUDY

	<20 years		21-40 years		41-60years		>60 years		Total	
	n	%	N	%	N	%	n	%	n	%
Male	1	20.0%	7	41.2%	21	42.0%	17	60.7%	46	46.0%
Female	4	80.0%	10	58.8%	29	58.0%	11	39.3%	54	54.0%
Total	5	100.0%	17	100.0%	50	100.0%	28	100.0%	100	100.0%
Statistical inference	X^2= 4.283 Df=3 p=0.233>0.05 Not Significant									

TABLE 4

OCCUPATION AS RISK FACTORS AMONG COPD PATIENTS

		COPD N=100	
		No	%
1	Bio gas inhalation	20	41%
2	Cotton mill worker	14	29%
3	Agricultural worker	10	20%
4	Rice mill worker	5	10%

Bio gas inhalation was associated with 41% of COPD, 29% in cotton mill worker

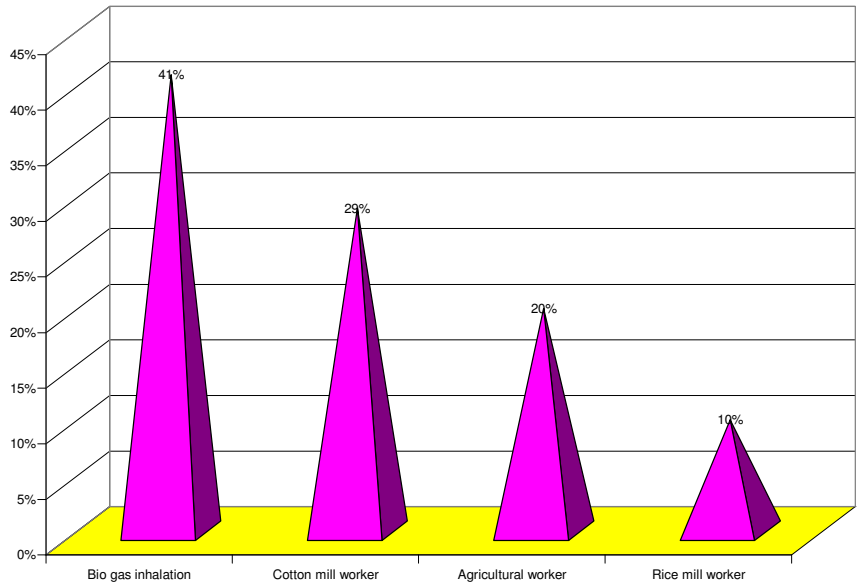


TABLE 5

RISK FACTORS ASSOCIATION OF COPD SMOKING AND ALCOHOL

NO		COPD	
		No	%
1	Smoking	23	26.45
2	Non Smoking	77	88.55
3	Smoking and Alcohol	15	17.25
		115	

TABLE 6

ANALYSIS OF CAUSES OF COPD IN PATIENTS

NO		COPD	
		No	%
1	Smoking	23	31
2	Bio gas inhalation	20	27
3	Smoking and Alcohol	15	21
4	Agricultural worker	10	14
5	Cotton mill worker	14	7

Charts

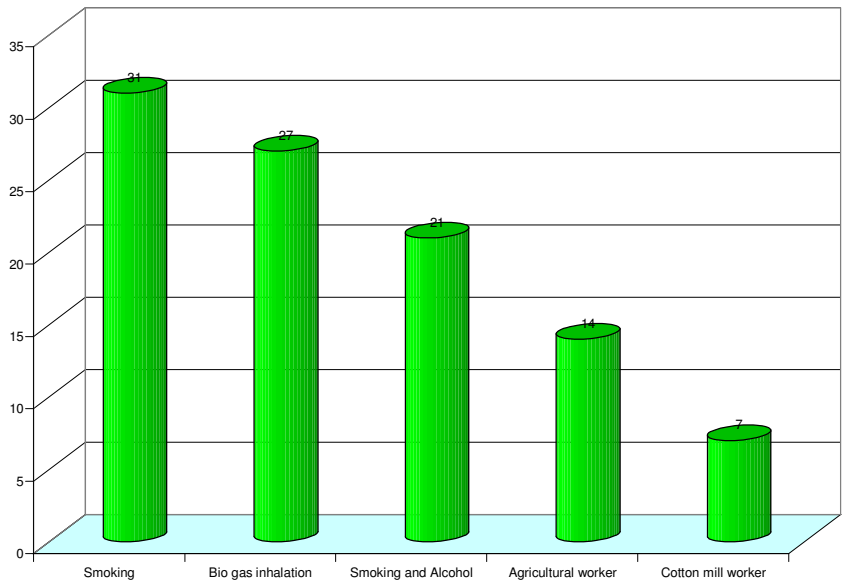


TABLE 7

CASE DISTRIBUTION AMONG COPD PATIENTS

NO	TYPE	NO	%
1	Chronic bronchitis	60	60
2	Emphysema	15	15
3	Bronchial asthma	25	25

Chronic bronchitis was the most common condition in COPD

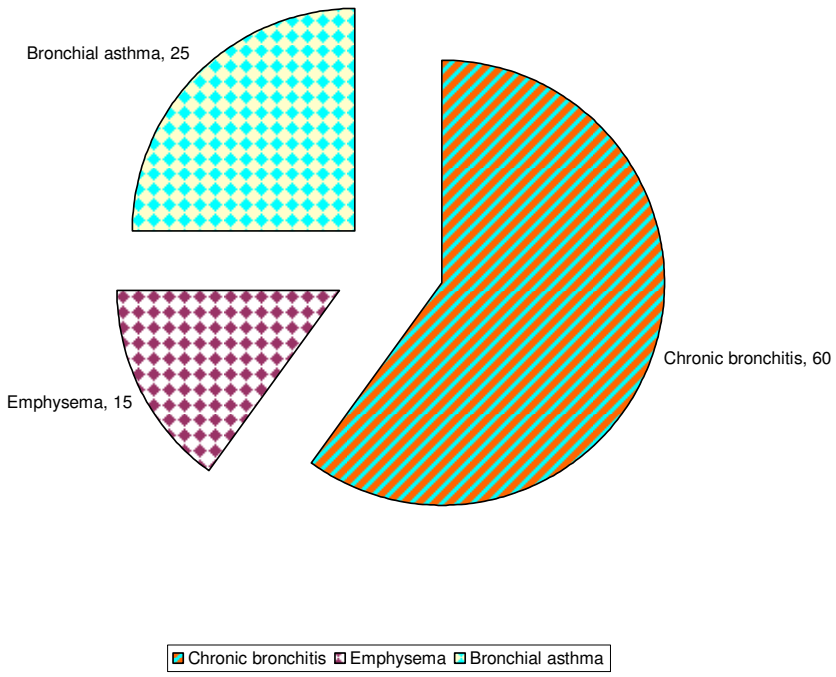


TABLE 8

SYMPTOMS ANALYSIS IN COPD

NO	SYMPTOMS	COPD	
		No	%
1	Shortness of breath	40	35
2	Cough	35	31
3	Cough with sputum production	30	26
4	Chest pain	5	4
5	Fever	5	4

Increased level of Cough with expectoration and breathlessness were the commonest symptoms among patients with COPD

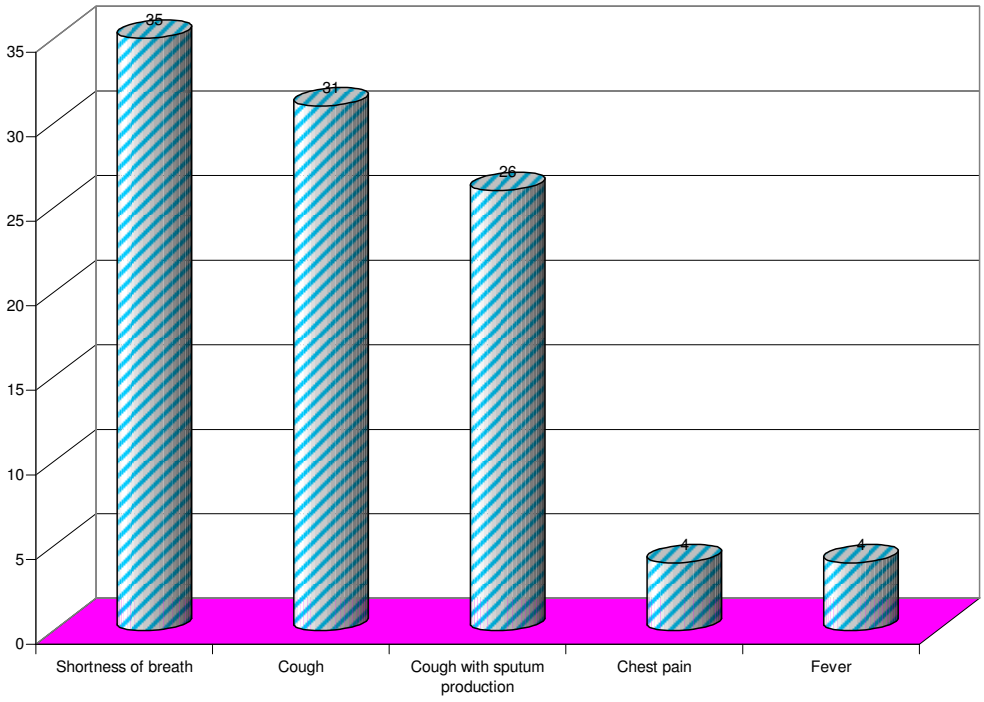


TABLE 9

COMORBID ILLNESS IN COPD

NO	Co morbid illness	COPD	
		No	%
1	DM	20	57
2	H T	10	29
3	CAD	5	14

DM was the most common Co morbid illness followed by Hyperion and CAD

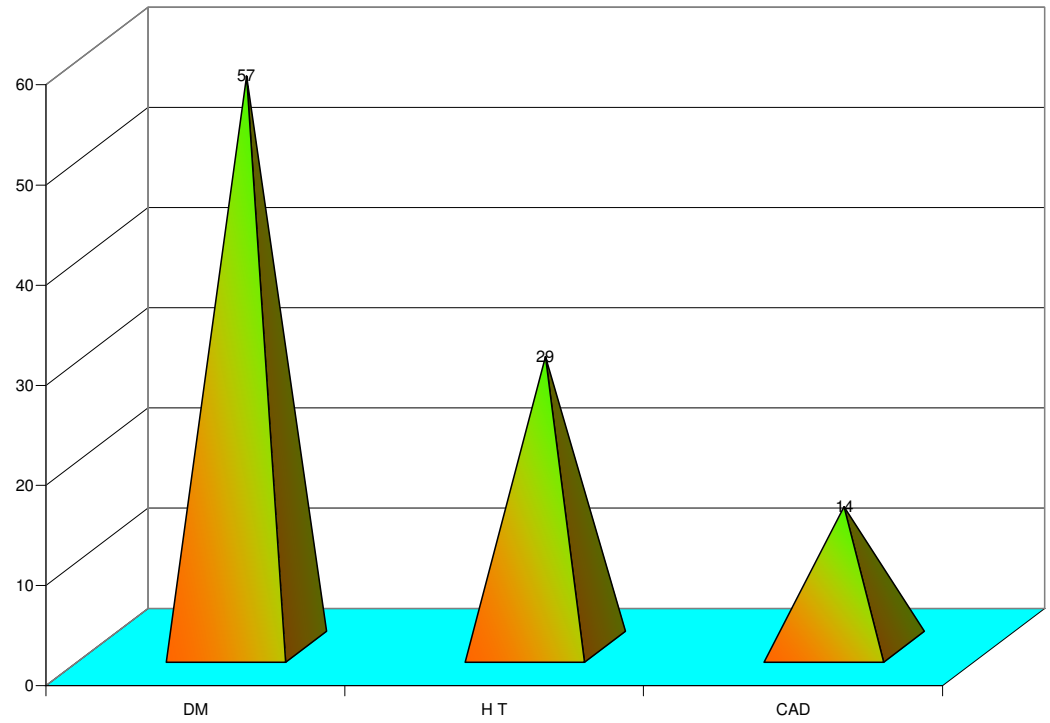


TABLE 10

COMPLICATIONS IN COPD PATIENTS

NO	Complications	COPD	
		No	%
1	Corpulmonale	6	46
2	Respiratory failure	4	31
3	Cardiac failure	3	23

Corpulmonale was the most common complication followed by respiratory failure

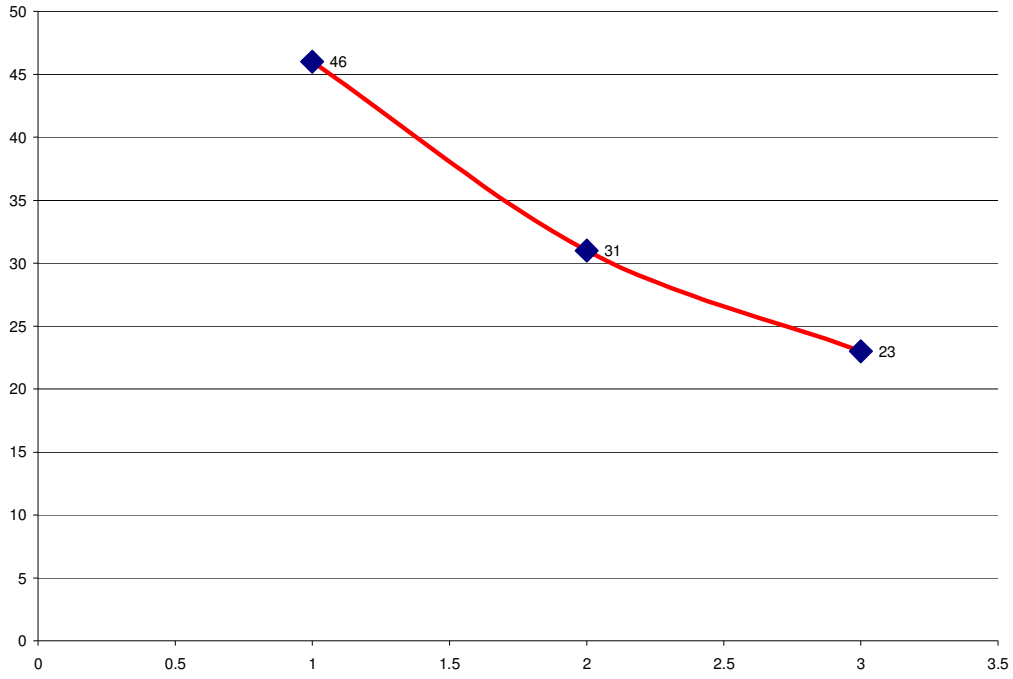


TABLE 11

PATHOGENS ISOLATED FROM COPD PATIENTS

NO	Pathogens Isolated	COPD	
		No	%
1	Bacteria	43	30
2	Fungi	18	13
3	Polymicrobial	10	7
	Total pathogens isolated	71	50

TABLE 12

TYPE AND COMBINATION OF PATHOGENS ISOLATED

NO	Type of Pathogens	No of cases N=100	%
1	Insignificant Growth	28	28
2	Monobacterial	43	43
3	Polymicrobial	10	10
4	Bacterial and Bacterial	7	7
5	Bacterial and Fungal	3	3

43% showed pure bacterial growth, mixed infection was seen in 10% of the cases

TYPE AND COMBINATION OF PATHOGENS ISOLATED

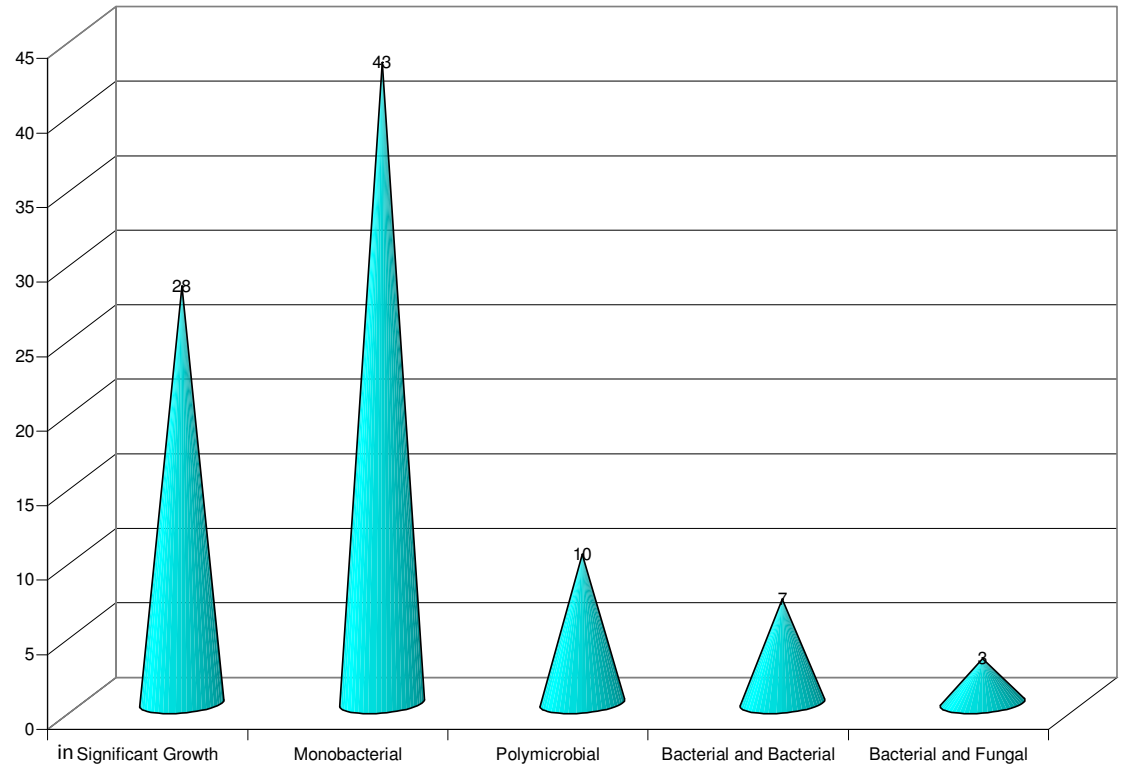


TABLE 13

BACTERIAL PATHOGENS ISOLATED FROM COPD PATIENTS

NO	Pathogens Isolated	No of Isolated	%
1	Klebsiella	16	37
2	Pseudomonas aeruginosa	12	27
3	Staphylococcus aureus	6	14
4	Escherichia coli	5	11
5	Moraxella Catarrhalis	1	2
6	Acinetobacter	4	9

Chart

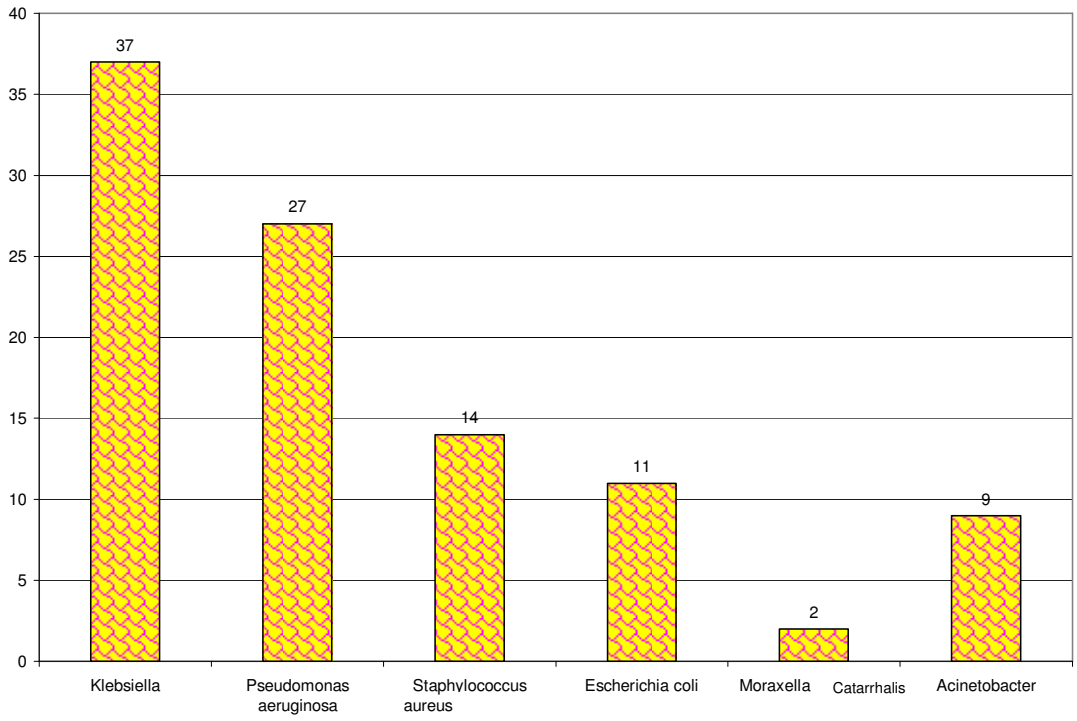


TABLE 14

BACTERIAL PATHOGENS ISOLATED FROM COPD PATIENTS

GRAM NEGATIVE BACTERIA

NO	Pathogens Isolated	No of Isolated	%
GRAM NEGATIVE BACTERIA			
1	Klebsiella	16	43
2	Pseudomonas aeruginosa	12	32
3	Escherichia coli	5	14
4	Acinetobacter	4	11
5	Moraxella Catarrhalis	1	10
GRAM POSITIVE BACTERIA			
1	Staphylococcus aureus	6	60
2	MRSA	3	30

TABLE 15

Fungal Pathogens Isolated FROM COPD PATIENTS

NO	Pathogens Isolated	No of Cases	%
1	Candida albicans	9	41
2	Aspergillus niger	5	23
3	Aspergillus flavus	4	18
4	Aspergillus fumigtius	4	18

TABLE 16

DRUG RESISTANCE MECHANISM AMONG THE PATHOGEN

ISOLATED FROM COPD PATIENTS

NO	Pathogens n=43	No of Drug resistant isolation	%
1	Klebsiella pneumoniae n(16)	9	28
2	ESBL	7	22
3	Amp C&β Lactamase	4	13
4	Pscudomonas aeruginosa n=(12) Multi drug resistant	6	19
5	Acinetobacter n=(4) Multi drug resistant	2	6
6	Moraxella Catarrahalis n=(1)	1	3
7	Methicillin resistant Staphylococcus aureas	3	9

TABLE 17

ANTIMICROBIAL SENSITIVITY PATTERN OF GRAM POSITIVE BACTERIA

NO	Antibiotics	Staphylococcus aureas	%
1	Amikacin	1	8
2	Amoxycillin Clavulanic acid	0	0
3	Ciprofloxacin	2	17
4	Erythromycin	1	8
5	Cefotaxime	2	17
6	Vanconycin	6	50

TABLE 18

ANTIMICROBIAL SENSITIVITY PATTERN OF GRAM NEGATIVE BACILLI

Antibiotics	E.coli n=5		Klebsiella pneumonia n=16		Pseudomonas n=12		Acinetobacter n=4	
Amikacin	2	40	12	75	8	66.7	2	50
Ceftazidime	-		4	25	2	16.7	2	50
Cefotaxime	1	20	4	25	3	25	2	50
Ciprofloxacin	1	20	10	62.5	8	66.7	2	50
Gentamycin	1	20	8	50	8	66.7	1	25
Imipenem	5	100	16	100	12	100	4	100
Piperacillin-Tazobactam	-	100%						

TABLE 19

DETECTION OF ESBL PRODUCERS AMONG THE ISOLATES

Pathogens	No of Positive Isolated					
	Screening Test		DDST		PCDDT	
	N	%	n	%	n	%
Klebsiella pneumonia n=16	9	56.25	7	43.75	7	43.75
E.coli n=5	2	40	2	40	2	40

TABLE 20

DETECTION OF AMPC PRODUCERS AMONG THE ISOLATES

Pathogens	No of Positive Isolates					
	Screening Test		DDST		PCDDT	
	N	%	N	%	n	%
Klebsiella pneumonia n=16	9	42.8	7	58	7	58
Pseudomonas n=12	8	38	4	33	4	33
Acinetobacter n=4	4	19	1	8	1	8

TABLE 21

DETECTION OF MBL PRODUCERS AMONG THE ISOLATES

Antibiotics	Pseudomonas n=4		Acinetobacter n=4	
	Senstive	Resistant	Senstive	Resistant
Imipenem	12(100%)	-	4(100%)	-
Imipenem EDTA	12(100%)	-	4(100%)	-

TABLE 22

DETECTION OF METHICILLIN RESISTANCE AMONG THE ISOLATES

Methods	Staphylococcus aureas n=(6)	
Disc diffusion method	3	50
MIC detection	3	50

FIGURE1 : SPUTUM CONTAINER

FIGURE 2 : COLONY COUNTER

FIGURE 3: PH METER

FIGURE 4: X RAY CHEST OF COPD PATIENTS

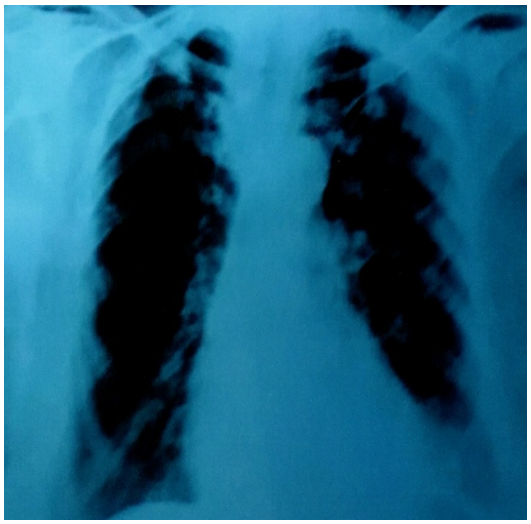
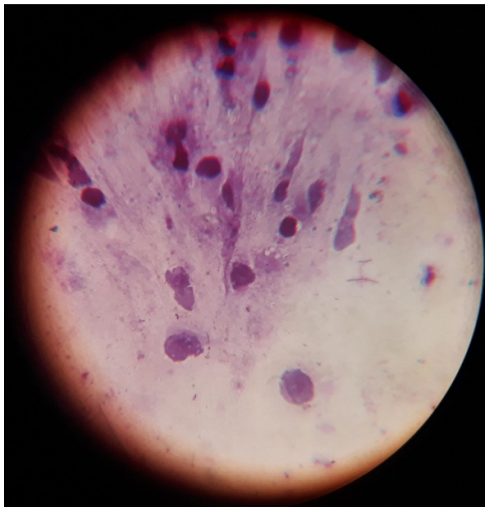


FIGURE 5:DIRECT GRAM STAIN FROM SPUTUM SAMPLE SHOWS PLENTY OF PUS CELLS GRAM NEGATIVE BACILLI



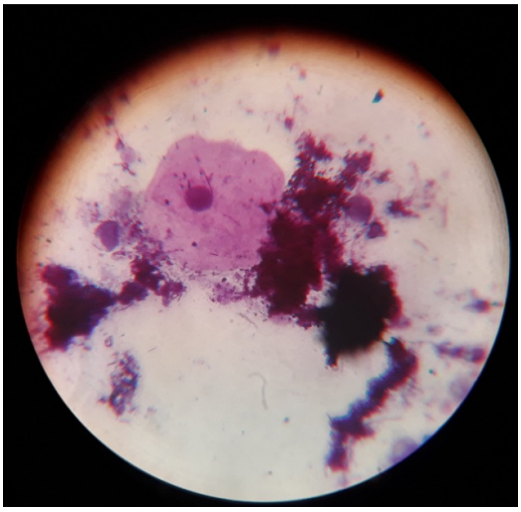


FIGURE 6: GRAM POSITIVE BACTERIA

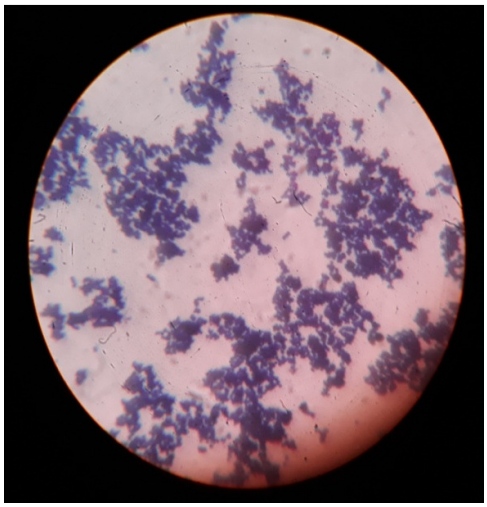
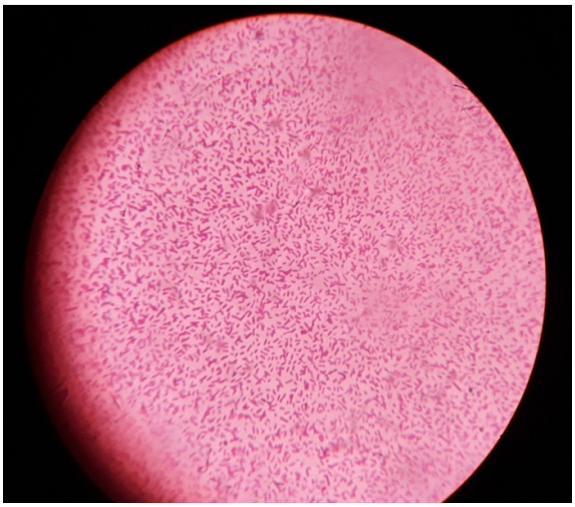


FIGURE 7: GRAM NEGATIVE BACTERIA

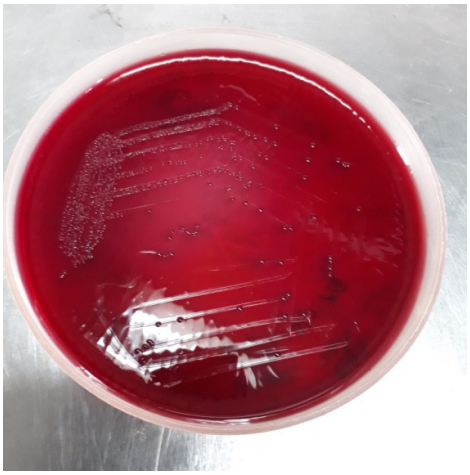


BACTERIAL IDENTIFICATION

FIGURE 8: LACTOSE FERMENTING COLONIES ON MACCONKEY PLATE .

STAPHYLOCOCCUS AUREUS

ESCHERICHIA COLI



KLEBSIELLA PNEUMONIAE

PSEUDOMONAS AEROGINOSA

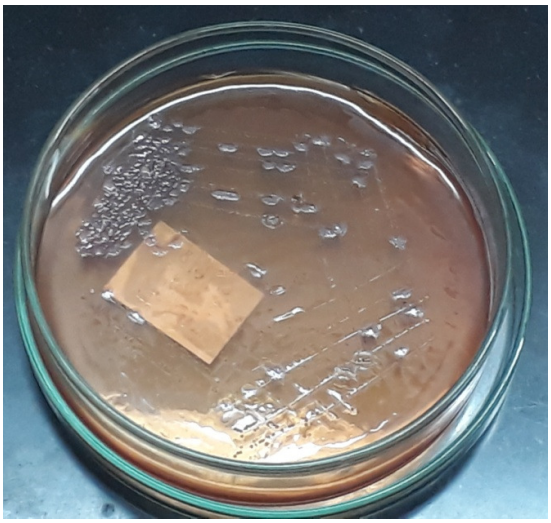


FIGURE 9 : COLONIES ON BLOOD AGAR PLATE

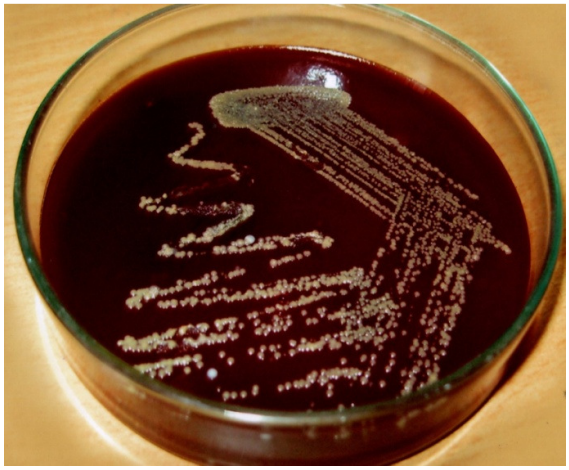
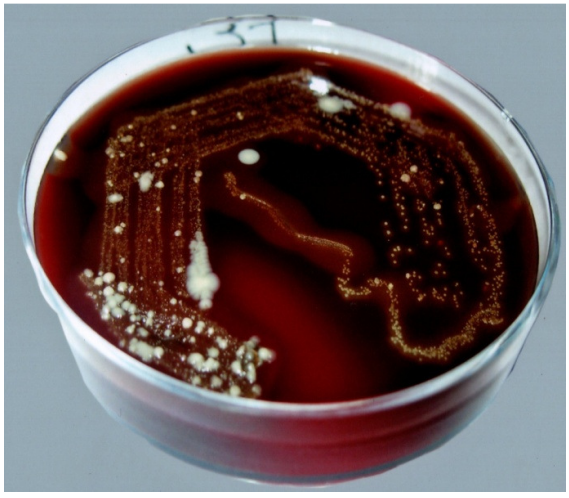


FIGURE 10 : POLYMICROBIAL PATHOGENS

:ON BLOOD AGAR PLATE



: ON MAC CONKEY PLATE

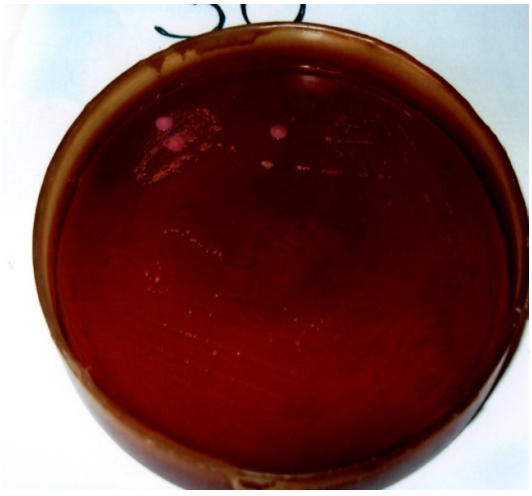
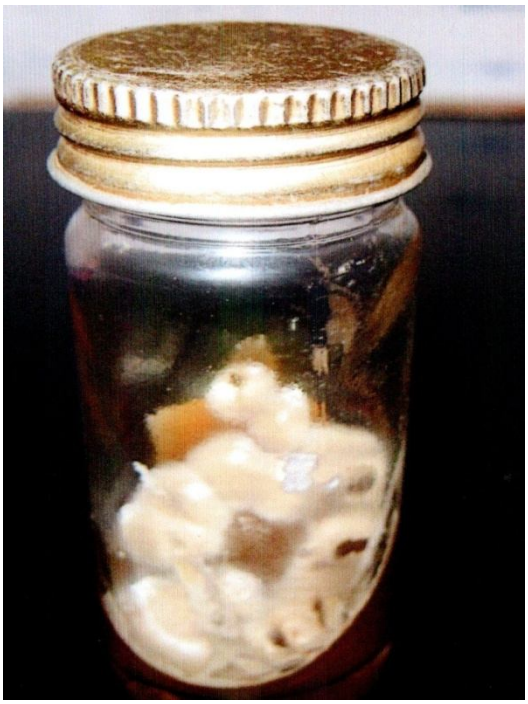


FIGURE 19:FUNGAL IDENTIFICATION

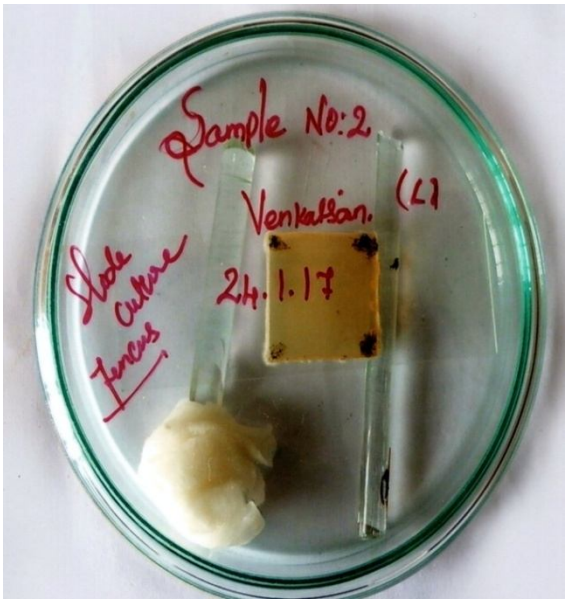
MACROSCOPIC APPEARENCE OF

:CANDIDA spp



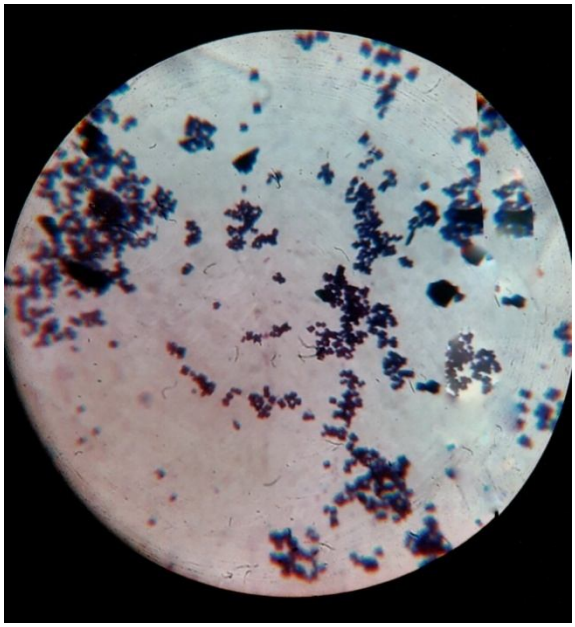
: ASPERGILLUS spp

SLIDE CULTURE TEST



MICROSCOPIC APPEARANCE

FIGURE 20 : CANDIDA ALBICANS



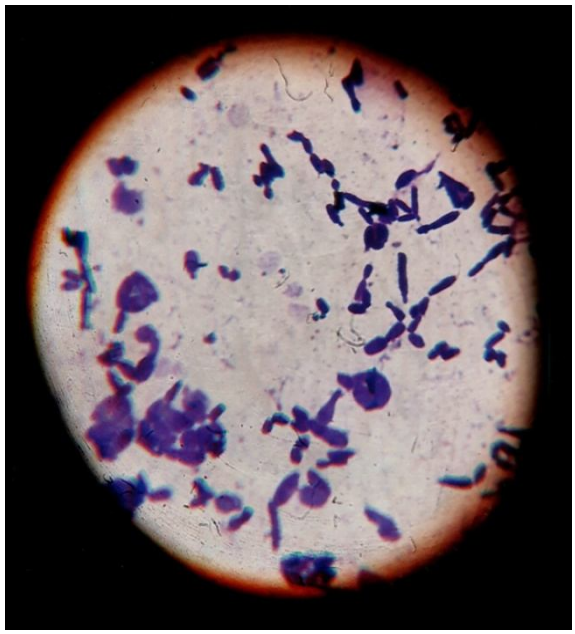
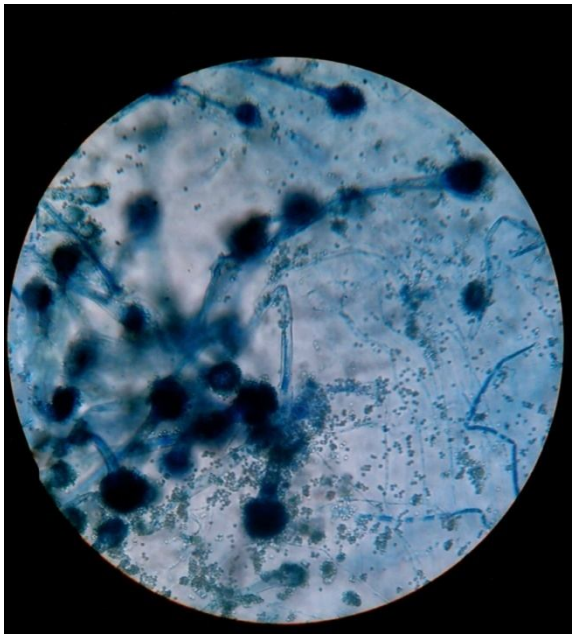
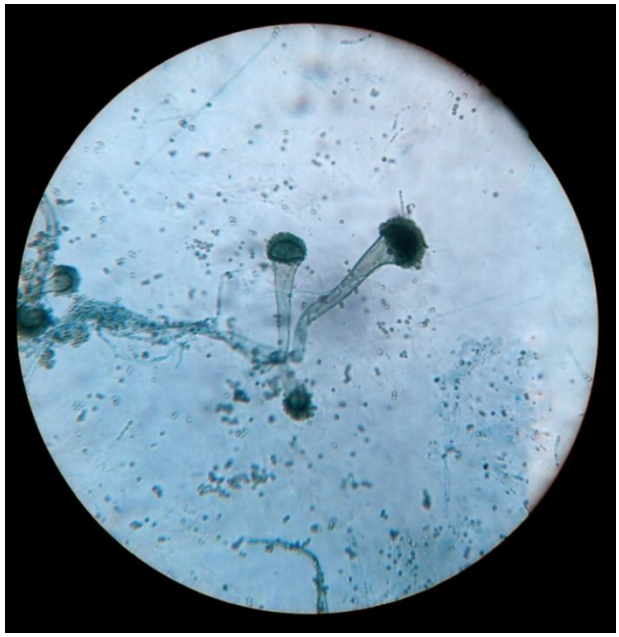


FIGURE 21 : ASPERGILLUS spp

A. FLAVUS

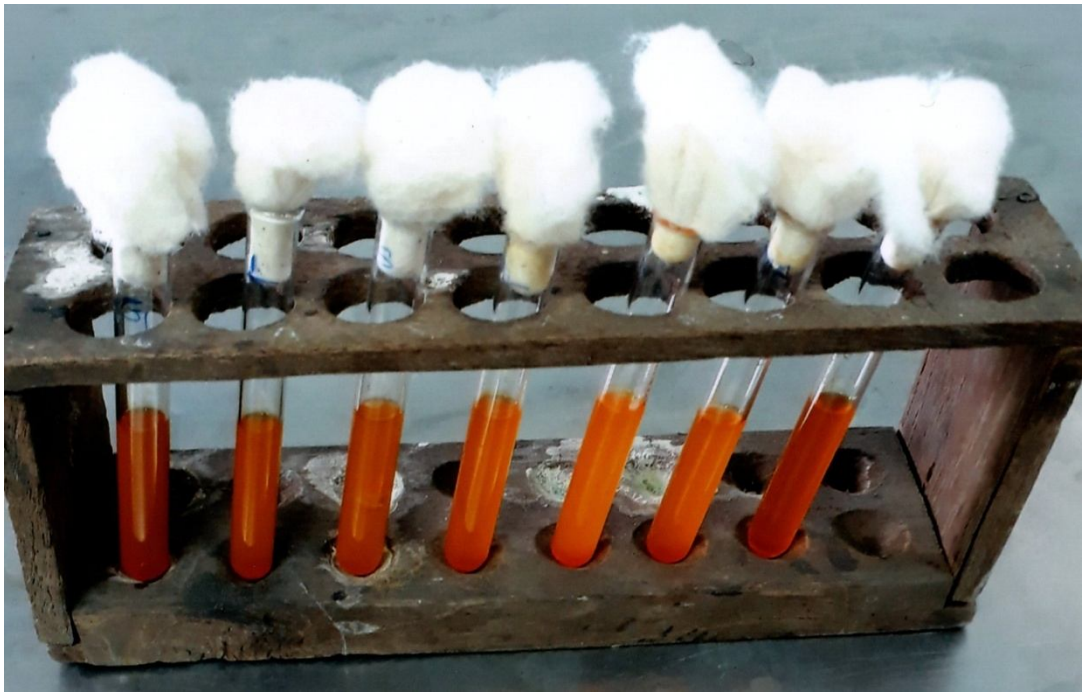


A. NIGER



BIOCHEMICAL REACTIONS

FIGURE 14 : SUGAR FERMENTATION TEST



FOR E.COLI FOR K.PNEUMONIAE FOR PSEUDOMONAS AERUGINOSA

FIGURE 15: OXIDASE TEST

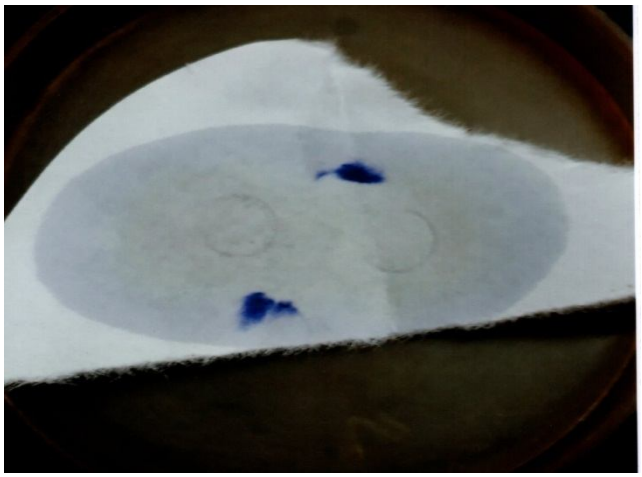


FIGURE 16: PPLO AGAR BASE

FIGURE 17: OF TEST

FIGURE 18: LAO TEST

DISCUSSION

This prospective study was conducted at Thanjavur Medical College Hospital in association with the Department of Thoracic Medicine at Thanjavur Medical College, Thanjavur. The study population consisting of 100 patients with signs of symptoms of COPD patients were selected according to GOLD Criteria[9]

The study population included in the COPD patients were in the age-group of 18-80 years. The majority of COPD patients in our study were in the age-group of 40-60 years (47%) Table – I

This can be explained by the fact that COPD has the highest prevalence after fifth or sixth decade of life. As age advances, the physiological decrease in lung function is accentuated because of the expression of deterioration in host defences at the bronchial mucosal level in patients with advanced age. This study observation correspond to the study of Jorg *et al.*

In our study COPD was more common among smokers. More than 50% in males were seen and were exposed more to outside environment. This was similar to the study done by Arora *et al*. Smoking causes an inflammatory process in the airways.

Smoking followed by biogas inhalation is a risk factor in female patients. There was a female predominance of 54% over males in COPD Among

women exposure to indoor air pollution was an important risk factor who were exposed to biomass fuel and air pollution resulting from the burning of wood and other biomass fuel –wood, charcoal, other vegetable matter and animal dung was also a risk factor in COPD. In our study 41% of biogas inhalation was seen in female patients. This study corresponds to the study of Lopez *et al*.

Occupation as a risk factor of COPD was found in 29% of cotton mill worker, in 20% agricultural workers and 10% of rice mill workers in this study.

Higher prevalence of chronic bronchitis was seen in this study as compared to emphysema[86]

Comorbidities –diabetes was found in 20% of COPDpatients.This was corresponding to a study done by Holguin [87]

Complications were observed in COPD patients. Corpulmonale was the most common complication (46%) followed by respiratory failure (31%) (Adil, Shujaat, *et al*)

Sputum was the most common sample collection processed from patients with COPD. Out of 100 samples 60% showed culture positivity and 40% showed culture negativity. Similar to the study of Groenewegan *et al*. A similar observation was made by Arora *et al* in which culture positive in

>60% of cases among all respiratory samples. Purulent sputum yielded positive bacterial culture as compared to nonpurulent sputum which correlates with the study done by Chawla K *et al.*

A mono Bacterial growth was identified in 43% while polybacterial growth was observed in 10% (Table 12). This is similar to the study of Gerard Rakesh *et al.*

In our study, the bacterial and fungal isolates from the COPD patients of varied etiology were isolated.

BACTERIAL ISOLATES

(1) Gram negative bacteria were the commonest organisms in the respiratory sample in patients with COPD. 80% as compared to 20% gram positive bacteria (Niederman Michael 2000) [92]

(2) Niderman and Michael have mentioned in their studies that gram negative bacteria was the commonest bacteria isolated.

(3) Among the gram negative organisms *klebsiella pneumonia 37%* was the common bacteria in our study, followed by pseudomonas *aeruginosa* 27%, *Esherichiacolli 11% and acinetobacter 9%*. This is similar to the study by Chawla et al and Mahebi *et al* [88,89]

(4) A study done by Eller jong *et al* which states the prevalence of pseudomonas with *acinetobacter* increases in patients with declining lung function. This present study observation corresponds to the study by N Roche *et al*.

(5) Polymicrobial infection among COPD patients was found in 10% of culture positive cases. This polymicrobial infection was seen with *klebsiella pneumonia* and pseudomonas aeruginosa followed by Ecoli and *acinetobacter*. Similar study by Gerard Rakesh *et al* reported polymicrobial infections in 6 cases of *klebsiella pneumonia*& 3 cases of pseudomonas *aeruginosa* and 3 cases of *acinetobacter* and staphylococcus which was similar to our findings.

FUNGAL ISOLATES

1. Fungal infections occur most often with other pathogens in immuno compromised patients. In the present study 22 fungal pathogens were isolated. In our study candida species 41 %, *Aspegillus niger* 23%, *Aspegillus flavus*18% and Aspegillusfumigatus18% were isolated.
2. These patients had repeated isolations of the above mentioned fungi with the sputum sample collected on three consecutive days. The gram stained smears also revealed pseudophyphae with yeast cells. Shanker *et al*[93] reported candida species in 80% of their isolates.

3. Shivananda[94] in his study in 1992 on 825 patients with pulmonary infection found 15% isolates were Aspergillus species.[95] Pulmonary Aspegillosis in 36 samples were documented by Geethalakshmi *et al*.[95]

POLYMICROBIAL ISOLATES

Polymicrobial isolates were found to be common in COPD patients. *Klebsiella pneumonia*, pseudomonas *aeruginosa, acinetobacter* and staphylococcus aureus were found to be more. This was similar to the study of Shanker *et al.*

ANTIBIOTIC SUSCECTIBILITY

1) The antibiogram was performed for various isolates and were analyzed. The commonest gram negative bacilli isolated from respiratory samples of COPD patients was *klebsiella pneumonia* that showed 75% sensitivity to amikacin, 62.5% sensitivity to ciprofloxacin.
    - 22% were extended spectrum β lactamase producing strains.
    - Similar study was observed by Gerard Rakesh *et al*[96].
    - Among the mechanisms of resistance to third generation Cephalosprin sproduction of ESBL and AmpC β lactamases are the most common.

- Out of the 16 isolates of klebsiella spp screened for ESBL production 56.25% found to be positive. By phenotypic confirmatory disc diffusion method 43.75% were confirmed as ESBL producers.
- Among the 16% isolates 42.8% were found to be AmpC producers. Hemalatha *et al* also showed AmpC production in 47.3% of isolates [97.]

2) Out of 12 isolates, pseudomonas *aeruginosa*was 66.7% sensitive to amikacin and ciprofloxacin were screened AmpC positive. 33% isolates were found to be AmpC producers by AmpC disc test. The overall rate of multidrug resistance in pseudomonas *aeruginosa* was 75%. However no isolate was found to be metallo-beta lactamase producers. This resistance pattern was similar to the study done by Chawla *et al* who stated 60% resistance of pseudomonas aeruginosa to third generation cephalosporins[98]

- Nonfermenter *acinetobactor baumannii* isolated from COPD cases showed 6% of multi drug resistant strains and showed a significant level of resistance to third generation cephalosporins and 8% were AmpC producers. This implies that *acinetobactor baumannii* was also one of the important drug resistant pathogens isolated from cases of COPD. This study is similar to the study by Wang Huei Sheng *et al* in 2013 where 15.3% of *acinetobactor baumannii* isolated from COPD patients were AmpC producers.

- All gram negative isolates in the study were found to be sensitive to imipenem, Khanfar *et al* in their study also showed all isolates were imipenem sensitive[99].
- The prevalence of carbapenamase producers varies depending upon clinical environment and use of inappropriate empirical antibiotic therapy.
- The most common gram positive isolates from respiratory samples in COPD patients in our study was staphylococcus aureus.
- 50% isolates of staphylococcus were found to be methicillin resistant. This indicates that the use of inadequate antibiotics during empirica ltherapy of longer duration of hospitalization may selectively enhance the growth of the drug resistant pathogen. A study conducted by INSAR group stated that the prevalence of MRSA in India varies from 25% in western part of India to 50% in South India. (Sangeetha *et al*) (2010). This study statement correlates with the present study. Chawla k *et al* in their study observed that 50% of Staphylococcus isolates were MRSA. All the MRSA isolates in our study were sensitive to vancomycin.

SUMMARY

This study was conducted at the Thanjavur Medical College Hospital. 100 patients admitted in Thoracic Medical Unit with various clinical presentations were studied .

- The majority of the COPD patients were in the age group 40-60 years.
- Among the patients in the study group, 75 showed symptoms of COPD.
- Sputum was the common specimen
- Chronic bronchitis was the common condition in COPD patients.
- Chronic bronchitis of 15 patients were emphysema.
- Smoking was associated with 60% risk among COPD patients.
- Biogas inhalation was associated with COPD.
- Most common symptoms were shortness of breath and cough with expectoration.
- Complications were observed in15% among which Corpulmonale was the most common complication followed by respiratory failure.
- Bacterial and fungal pathogens were isolated. Among the bacteria, gram negative bacilli *klebsiella pneumoniae* was the most commonly isolated bacilli
- Among the fungal pathogens identified, *Candida* and *Aspergillus spp* were the common one in this study.
- *Mycoplasma* was not isolated in this study.

CONCLUSION

This study on 100 patients admitted in Thoracic Medical Unit, Thanjavur Medical College, Thanjavur was conducted in order to study the risk factors of COPD, etiological agents, antibacterial susceptibility pattern of the isolates and prevalence of multi drug resistance among the isolates.

Exacerbations mostly of infective etiology are a frequent cause of morbidity in COPD patients. So this study has been taken to analyze the bacterial and fungal profile with their sensitivity pattern. Bacterial infections are the most common reason for exacerbations, in our study among which *Klebsiella pneumoniae and pseudomonas aeruginosa* were the commonest.

Purulent sputum sample is a good and easy to obtain, that provides preliminary idea about the pathogen, thereby helping in selecting antibiotic for empirical antibiotic therapy.

Hence the choice of empiric antibiotics should be based on local pathogens prevalence and antibacterial susceptibility and on identification of patients with selected clinical parameters at high risk of developing infections caused by multidrug resistant micro organisms. Hence periodic isolation and identification of resistant status of pathogens responsible for COPD will be helpful in formulating the antibiotic policy.

Continuous surveillance to detect the resistant strains, strict guide lines for the antibiotic therapy and the implementation of infection control measures reduce the increasing burden of antibiotic resistance.

A knowledge of the resistant pattern of ESBL producing *Enterobactericiae* in the geographical area will help us to formulate appropriate treatment protocol which will be of immense use in producing mortality and morbidity.

Along with conventional antibiogram, routine ESBL testing should be done which reduces the volume of antibiotics and development of resistance to antibiotic.

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
1	60	F	443	sputum	COPD/AE	Escheria Coli	NIL	-	Biomass	-	-
2	60	M	54537	sputum	Chronic Bronchitis	Klebsiella pneumoniae	NIL	-	-	AW	-
3	60	F	2234	sputum	BA/AE	Klebsiella pneumoniae	NIL	smoker alcoholic	Biomass	-	-
4	50	M	3208	sputum	COPD/AE	-	NIL	smoker alcoholic	Biomass	-	-
5	36	M	3028	sputum	Chronic Bronchitis	Klebsiella pneumoniae	NIL	smoker alcoholic	Biomass	-	-
6	65	F	1649	sputum	BA/AE	Escheria Coli	HT	-	Biomass	-	-
7	35	F	4330	sputum	Chronic Bronchitis	Pseudomonas aeruginosa	NIL	-	Biomass	-	-
8	47	F	4067	sputum	Chronic Bronchitis	NF	NIL	-	Biomass	AW	-
9	65	F	4366	sputum	COPD / AE	Pseudomonas aeruginosa	NIL	-	Biomass	-	-
10	60	F	4566	sputum	Chronic Bronchitis	-	DM	smoker alcoholic	-	-	-
11	53	F	4594	sputum	BA/AR	-	NIL	-	Biomass	-	-
12	56	F	5477	sputum	COPD	Pseudomonas aeruginosa	NIL	-	Biomass	-	-
13	18	F	5233	sputum	Chronic Bronchitis	-	-	-	-	-	-
14	45	F	6484	sputum	COPD	Aspergillus flavus	RVF/(CF)	-	Biomass	-	-
15	45	M	6114	sputum	COPD	-	-	smoker alcoholic	-	-	CMW
16	32	M	6084	sputum	Chronic Bronchitis	-	HT	Alcoholic	-	-	CMW

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
17	63	M	8626	sputum	COPD	Staphylococcus	HT	-	-	-	RICE MILL WORKER
18	40	M	8798	sputum	BA	Staphylococcus	-	smoker		-	CMW
19	75	M	9837	sputum	COPD	Klebsiella pneumoniae	-	-	-	-	CMW
20	62	M	11010	sputum	COPD/AE	Pseudomonas aeruginosa	-	-	-	-	CMW
21	57	M	9003	sputum	COPD	-	DM	-	Biomass	-	
22	46	M	11173	sputum	COPD/AE	Aspergillus flavus	-	alcoholic smoker	Biomass	-	
23	57	M	11198	sputum	BA/AE	Escheria Coli	-	-	-	-	
24	60	M	12327	sputum	BA/AE	Acinetobacter baumannii	-	-	-	-	
25	60	M	10439	sputum	Chronic Bronchitis	Pseudomonas aeruginosa	-	-	-	AW	
26	35	Metc	15073	sputum	Chronic Bronchitis	-	-	alcoholic smoker	-	-	RICE MILL WORKER
27	37	F	16075	sputum	BA/AR	-	-	-	-	-	CMW
28	25	F	15258	sputum	Chronic Bronchitis	-	-	-	Biomass	-	-
29	45	F	12726	sputum	COPD	Acinetobacter baumannii	-	-	-	AW	
30	57	F	16465	sputum	BA/AR	Pseudomonas aeruginosa	-	-	-	-	RICE MILL WORKER
31	43	F	16843	sputum	BA/AE	Escheria Coli	-	-	Biomass	-	
32	18	F	16850	sputum	Chronic Bronchitis	Aspergillus niger	-	-	-	-	

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
33	60	F	15572	sputum	Chronic Bronchitis	-	HT/DM	-	-	-	0
34	56	F	17954	sputum	COPD	Acinetobacter baumannii	-	-	Biomass	-	
35	36	F	17699	sputum	COPD	-	-	-	Biomass	-	
36	42	M	18536	sputum	COPD/AE	Candida Pseudomonas	-	smoker alcoholic	-	AW	-
37	56	M	18576	sputum	COPD	-	-	smoker	-	-	STEEL MILLWORKER
38	55	F	18872	sputum	BA/AE	Pseudomonas aeruginosa	-	-	-	AW	
39	49	M	19694	sputum	COPD/AE	-	-	Betal Leaf chewer	-		STEEL MILLWORKER
40	52	M	20706	sputum	COPD/AE	-	-	smoker	-	AW	
41	44	F	20412	sputum	COPD	Acinetobacter baumannii	-	-	Biomass		
42	75	F	20500	sputum	BA/AE	-	-	-	Biomass	-	
43	54	Metc	22125	sputum	COPD	-	-	smoker		-	
44	75	M	26734	sputum	Chronic Bronchitis	-	-	-	-	-	
45	35	F	22131	sputum	BA / AE	-	-	-	Biomass	-	
46	23	F	21999	sputum	COPD	Aspergillus niger	-	-	Biomass	-	
47	47	F	138433	sputum	COPD	Pseudomonas aeruginosa	-	-	-	-	
48	40	F	24637	sputum	COPD	Candida Pseudomonas	-	-	-	-	

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
49	15	F	24051	sputum	COPD	Candida	-	-	-	-	
50	34	F	24635	sputum	COPD	Pseudomonas aeruginosa	-	-	-	-	
51	37	F	25848	sputum	COPD	-	-	-	-	-	
52	27	F	24896	sputum	COPD	-	-	-	Biomass	-	
53	45	F	25858	sputum	COPD	Pseudomonas aeruginosa	-	-	Biomass	-	
54	42	M	25073	sputum	Chronic Bronchitis		-	-	Biomass	-	
55	72	F	25500	sputum	COPD	-	CAD	-	-	AW	
56	61	M	25272	sputum	COPD	-	-	smoker	-	-	
57	54	M	26316	sputum	COPD / AE	-	-	-	-	-	
58	70	M	26025	sputum	COPD	Pseudomonas aeruginosa	DM	smoker alcoholic	Biomass	-	
59	49	F	28023	sputum	COPD	-	-	-	Biomass	-	
60	60	F	27335	sputum	COPD	-	CAD	-	Biomass	-	
61	46	F	20224	sputum	COPD	-	Allergic disorder	-	-	AW	
62	56	M	29130	sputum	COPD	-	HT/DM	-	-	-	RICE MILL WORKER
63	18	M	30368	sputum	BA/AE	-	-	-	-	-	-
64	52	F	29432	sputum	COPD	Staphylococcus	DM	-	Biomass	-	

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
65	65	M	31496	sputum	COPD/AE	-	Cardiac failure	Smoker	-	-	COTTON MILL WORKER
66	82	M	31158	sputum	COPD/AE	Staphylococcus	Respiratory failure	-	-	-	COTTON MILL WORKER
67	70	F	32069	sputum	COPD/AE	Staphylococcus	DM		Biomass	-	
68	40	M	32004	sputum	COPD	-	DM	smoker	-	-	COTTON MILL WORKER
69	61	M	33816	sputum	COPD/AE	-	DM	smoker	-	AW	
70	61	M	33080	sputum	COPD/AE	-	DM	smoker	-	-	RICE MILL WORKER
71	20	F	32273	sputum	Acute Severe Asthma	-	Allergic disorder		-	-	
72	65	F	32829	sputum	COPD	-	DM /HT		-	-	COTTON MILL WORKER
73	55	M	32830	sputum	COPD	Pseudomonas aeruginosa	DM / Corpulmonale		-	AW	
74	78	M	31926	sputum	COPD	-	Corpulmonale	smoker alcoholic	-	AW	
75	45	M	31696	sputum	Chronic Bronchitis	Staphylococcus	HT	smoker alcoholic	-		
76	43	M	33269	sputum	Chronic Bronchitis	-	HT	smoker alcoholic	-	AW	
77	61	M	33592	sputum	Chronic Bronchitis	-	DM	smoker	-		cotton mill worker
78	63	F	33485	sputum	COPD /AE	-		smoker	-	AW	
79	60	M	33268	sputum	COPD	-	HT		-		
80	80	F	31905	sputum	COPD	Pseudomonas aeruginosa	Corpulmonale		-	AW	

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
81	66	F	50352	sputum	COPD/AE	Klebsiella pneumoniae	DM/AR	Smoker	-	AW	
82	50	F	49970	sputum	COPD	Staphylococcus	DM/AR	smoker	-	AW	
83	70	M	49758	sputum	COPD	-	Respiratory failure		-	AW	
84	45	M	49988	sputum	COPD /AE	-	Corpulmonale	smoker	-	AW	
85	28	M	50994	sputum	BA	-	Allergic disorder		-		
86	68	M	51006	sputum	COPD / AE	Pseudomonas aeruginosa	Corpulmonale / HT/DM	smoker	-	AW	
87	60	F	51235	sputum	COPD	-	Corpulmonale / HT/DM	-	-	AW	
88	65	F	57278	sputum	COPD	-	-	-	-	AW	
89	65	F	49629	sputum	COPD	Pseudomonas aeruginosa	-	-	-	AW	
90	60	F	49738	sputum	Chronic Bronchitis	-	Respiratory failure	-	Biomass	AW	
91	60	M	50978	sputum	COPD	Klebsiella pneumoniae	Cardiac failure	smoker Alcoholic	-		steel mill worker
92	70	M	53042	sputum	COPD / AE	Klebsiella pneumoniae	-	smoker Alcoholic	-		
93	59	M	53116	sputum	COPD/AE	Klebsiella pneumoniae	-	smoker Alcoholic	-		
94	50	M	54531	sputum	COPD	Klebsiella pneumoniae	-		-		
95	70	M	53328	sputum	COPD/AE	-	CAD	smoker	-		CMW
96	70	M	53328	sputum	COPD	Klebsiella pneumoniae	Cardiac failure DM/HT	Betal Leaf chewer	-	AW	

S.NO	AGE	SEX	IP NO	SAMPLE	DIAGNOSIS	ISOLATES	CO MORBIDITY AND COMPLICATIONS	SMOKER	BIO MASS FUEL	ARICULTURAL WORKER	COTTON MILL WORKER
97	77	METE	51697	sputum	COPD	Klebsiella pneumoniae	corpulmonale	Smoker	-		
98	30	M	54202	sputum	BA/AE	Klebsiella pneumoniae	-	smoker alcoholic	Biomass		
99	56	F	54162	sputum	COPD	Pseudomonas aeruginosa	-		Biomass		
100	57	M	53557	sputum	Chronic Bronchitis	-	HT/DM	smoker alcoholic	-		CMW

APPENDIX – I

ABBREVIATION

ATS	American Thoracic study
AECOPD	Acute exacerbation of Chronic obstructive pulmonary Disease
ATCC	American Type Culture Collections
CLSI	Clinical &Laboratory Standards Institute
COPD	Chronic Obstructive Pulmonary Disease
DDST	Double Disk Diffusion Synergy Test
ESBL	Extended Spectrum Beta lactamases
ERS	European respiratory Society
FEV1	Forced Expiratory Volume
FVC	Forced Vital Capacity
GNB	Gram–Negative Bacilli
GPC	Gram–Positive Cocci
MDR	Multi Drug Resistance
MHA	Muller Hinton Agar
MBL	Metallo ß - Lactamases
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant Staphylococcus aureus
MSSA	Methicillin Sensitive Staphylococcus aureus
PCDDT	Phenotypic Confirmatory Disk Diffusion Test

APPENDIX – II

1. STAINS AND REAGENTS

Gram Staining: -

a. Methyl violet (2%)	10g Methyl violet in 100ml absolute alcohol in 1 litre of distilled watter (Primary stain)
b. Gram's iodine	10g lodine in 20g KI (Fixative)
c. Acetone	Decolourizing agent
d. Carbol fuchsin 1%	Secondary stain

2. ACID FAST STAIN

Basic fuchsin powder	5 g
Phenol (crystalline	25 g
Alcohol (95% (or) 100% ethanol)	50 ml
Distilled Water	500 ml

20% Sulphuric acid

Conc Sulphuric acid (98%)	250 ml
Distilled Water 1L	

Methylene Blue	**Counter stain**
1 % Methylene	blue

3. 10% KOH

Potassium hydroxide	10g
Glycerol	10ml
Distilled Water	80 ml

4. LACTO PHENOL COTTON BLUE STAIN:-

Lactic acid	20 ml
Phenol	20 ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml

5. DIENE'S STAIN:-

Methylene blue	2.5 gm
Azure blue	1.25 gm
Maltose	10.0 gm
$Na_2 Co_3$	0.25 gm
Distilled water	100ml

B.MEDIA USED

1. MAC CONKEY AGAR

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00mI

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. BLOOD AGAR (5%SHEEP BLOOD AGAR)

Peptone	l0g
Nacl	5g
Distilled water	1Ltr
Agar	10g

3. SABOURAUD'S DEXTROSE AGAR

Dextrose	40g
Peptone	l0g
Agar	20g
Distilled water	l000ml

pH=5.5

4. PPLO AGAR

Peptone	10g
Meat infusion agar #	250
Sodium Chloride	5g

Final Ph (at 25°C)-7.8 ± 0.2

5. MUELLER-HINTON AGAR

Beef infusion	300ml
Caesein hydrolysate	17 .5g
Starch	1.5g
Agar	10g
Distilled water	1ltr

pH=7.4

Sterilise by autoclaving at 121° C for 20mins

C.MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenelene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Indole test

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	1g
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1ltr
10% sterile solution of glucose	l0ml
Sterile 20%urea solution	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue(0.2%)	40ml

Dispense, auto clave' at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef Extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	l2ml
Distilled water	1ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1ltr
Glucose10%solution	50ml

Dissolve the peptone and phosphate and adjust the Ph to 7.6. Filter dispense in 5ml amounts and sterilize at 121°c for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1%

Indicator

Bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar	1ml
Dislilled water	l00ml

pH=7.6.

9. Mannitol motility medium

Agar	5g
Peptone	1g
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	l000ml

pH=7.2

10. Potassium nitrate broth

Potassium nitrate (KN03)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5ml amount and auto claved.

11. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium hydrogen phosphate	l g
Sodium Chloride	5 g
Agar	12g
Distilled water	1 lr

pH=7.4

Distributed in tube sand sterilized by autoclaving at121°C for 15 minutes, allowed to solidify as long slopes.

12. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10ml
Sugar to be tested	20g
Water	1litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100°C for 30min on 3 consecutive days.

13. Sugar assimilation agar

Basal medium I
Yeast nitrogen base(Difco)
Agar
Water

Steam to dissolve and dispense in 10ml amounts in universal containers. Autoclave at 115° C for 15min.

ANNEXURE II

PROFORMA

Name	S. No.
Father's name	IP No
Age	Date of sample collection
Sex	Specimen
Address	Test
Family income	
Chief complaints	
Past history	
Personal history of smoking	
Occupational history	
History of Co-Morbid Illness	
Family history	

CLINICAL DIAGNOSIS

PHYSICAL EXAMINATION

ANNEXURE III

CONSENT FORM

STUDY TITLE

"A STUDY OF MICROBIOLOGICAL PROFILE IN OBSTRUCTIVE PULMONARY DISEASE IN A TERTIARY CARE HOSPITAL IN THANJAVUR"

I ……..hereby give consent to participate in the study conducted by Dr. M. Lidwin Mary, Post Graduate in Thanjavur Medical College, Thanjavur to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the diseases. I also give consent to give my sputum for further investigations. I also learn that there is no additional risk in this study. I also give my consent to the investigator to publish the data in any form or journal.

Signature/Thumb impression Place:

Of the patient/relative Date

Name of patient & Address

Signature of investigator

Signature of Guide

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